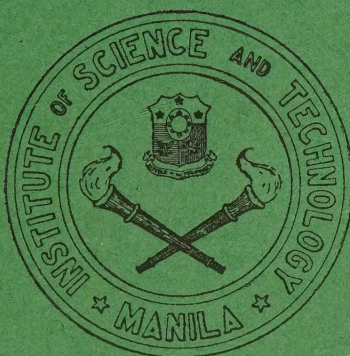


37/C
Vol. 86, No. 3

SEPTEMBER, 1957

THE PHILIPPINE JOURNAL OF SCIENCE



MANILA
BUREAU OF PRINTING
1958

76132



THE PHILIPPINE JOURNAL OF SCIENCE

Published by the Institute of Science and Technology
(Formerly Bureau of Science)

[Entered as second-class matter at the Post Office at Manila, Philippines]

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THE PHILIPPINE JOURNAL OF SCIENCE

VOL. 86

SEPTEMBER, 1957

No. 3

SCREENING OF PHILIPPINE PLANTS FOR STEROIDAL SAPOGENINS, II

BY FELICIDAD ESTORES ANZALDO, JOAQUIN MARAÑON,
and SANTA F. ANCHETA

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Steroidal sapogenin, an aglycon with a steroid nucleus known to occur in plants in a combined glycosidal form, is of value in the synthesis of cortisone. Because they are easily available and comparatively cheap, plants are a vast potential source of this substance. In the first part of this paper(1) mention was made of the importance of this investigation and previous researches independently conducted abroad and the results thereof were cited.

This paper covers the screening of Philippine plants under the families Solanaceæ, Amaranthaceæ, Moraceæ, Sapotaceæ, Verbenaceæ, Zingiberaceæ, Gramineæ, Meliaceæ, Tiliaceæ, Araceæ, Anonaceæ, Myrtaceæ, Palmæ, Rutaceæ, Rubiaceæ, Cucurbitaceæ, and Bixaceæ.

EXPERIMENTAL PROCEDURE

The different parts of the plants used in this study, which were collected from different places in the Philippines and identified according to families, genera and species, were dried, powdered, and weighed.

In all our experiments, a 10-gram sample of dried material was used. The detection and isolation of the steroidal sapo-

genins followed closely the procedure of Wall, et al.(7) and the estimation of the same was patterned after the method of Walens and his associates.(6) For extraction, 80 per cent ethyl alcohol was used as solvent. The alcoholic extracts were tested for hemolysis with standardized cow's blood suspended in normal saline solution. Digitonin solution was used as the standard. If complete hemolysis had occurred, the tube containing digitonin would be entirely clear. This was checked by centrifuging. The hemolyzed solution gave little or no red precipitate. Positive hemolysis test was confirmed with the aid of a microscope.

Samples giving positive hemolytic tests were acid hydrolyzed and extracted with benzene. The benzene extract was then acetylated, yielding a crude residue of sapogenin acetate. A yield of crude acetate less than 10 mg. meant less than 0.1 per cent pure sapogenin, and the sample was regarded as negative.

For the qualitative determination of the sapogenins, the spectra of the sulfuric acid chromogens were used. Spectral curves were determined by means of a Beckman DU spectrophotometer.

Details of the procedure on blood standardization, hemolysis test, isolation of crude sapogenin acetates adsorption chromatography, and spectrophotometric determination were described in the previous paper.

RESULTS AND DISCUSSION

Table 1 shows the results of the tests made on specific parts of plant species covered by this investigation. Fifty-three alcoholic extracts which gave positive results in the hemolysis test were isolated for their crude sapogenin acetates. Twenty-seven of these crude acetates dissolved in 94 per cent sulfuric acid solution gave characteristic ultraviolet spectral curves of the steroidal sapogenin. Most of these came from plants under the families Solanaceæ, Verbenaceæ, Amaranthaceæ and Bixaceæ, Plant samples investigated under the families Tiliaceæ, Sapotaceæ, Palmæ, Gramineæ, and Araceæ were found to be negative.

Identification was made by comparison of the absorption maxima of the different steroidal sapogenins given in Table 2. The estimation of the quantity of steroidal sapogenin content of the plant sample was made at 250 m μ .

TABLE 1.—Results of hemolysis tests and estimated sapogenin contents of plants.

Species	Local name	Plant part	Hemo- lysis test	Esti- mated total m.f.b.
SOLANACEÆ				Per cent
<i>Capsicum annuum</i> Linn. var. <i>longum</i> Sendt.	Sileng-haba	Fruit	+	0.68
<i>Capsicum frutescens</i> Linn. Sendt.	Sileng-labuyo	Fruit	+	0
<i>Capsicum annuum</i> Linn. var. <i>grossum</i> Sendt.	Sileng-bilog	Fruit	—	0
Do	do	Leaves	—	0
<i>Cestrum nocturnum</i> Linn.	Dama de noche	Leaves	+	0
Do	do	Stems	+	0.51
<i>Datura metel</i> Linn.	Talong-punai	Stems	—	0
Do	do	Leaves	+	0
<i>Lycopersicum esculentum</i> Mill.	Kamatis	Leaves and stems	—	0
<i>Nicotiana tabacum</i> Linn.	Tabako	Fruit	—	0
Do	do	Leaves	—	0
Do	do	Stems	+	0.16
<i>Solanum indicum</i> Linn.	Talong-na-puti	Fruit	—	0
<i>Solanum melongena</i> Linn.	Talong	Fruit	—	0
<i>Solanum nigrum</i> Linn.	Kunti	Leaves	—	0
<i>Solanum tuberosum</i> Linn.	Patatas	Tubers	+	0
<i>Solanum verbascifolium</i> Linn.	Malatalong	Leaves	+	0
Do	do	Stems	—	0
AMARANTHACEÆ				
<i>Amaranthus spinosus</i> Linn.	Urai	Whole plant	+	0.23
<i>Amaranthus viridis</i> Linn.	Kolitis	Whole plant	+	0
<i>Amaranthus paniculatus</i> Linn.	Halom	Whole plant	+	0.05
<i>Celosia cristata</i> Linn.	Palong-manok	Leaves	+	0
Do	do	Flowers	—	0
<i>Celosia argentea</i> Linn.	Kadayohan	Whole plant	+	0.27
MORACEÆ				
<i>Allaeanthus luzonicus</i> (Blanco) F. Vill.	Himbabao	Leaves	+	0.23
Do	do	Stems	—	0
<i>Artocarpus blancot</i> Merr.	Antipolo	Leaves	—	0
Do	do	Stems	—	0
Do	do	Bark	+	0.17
<i>Artocarpus camansi</i> Blanco	Kamansi	Stems	—	0
Do	do	Leaves	—	0
<i>Artocarpus incisus</i> Linn.	Rimas	Fruit	—	0
Do	do	Stems	—	0
Do	do	Leaves	—	0
<i>Artocarpus cumingiana</i> Trec.	Anubing	Leaves	—	0
Do	do	Stems	—	0
Do	do	Bark	—	0
<i>Artocarpus heterophyllus</i> Lam.	Langka	Leaves	+	0.07
Do	do	Stems	+	0.34
Do	do	Bark	—	0
<i>Ficus haurii</i> Blanco	Haurii	Leaves	+	0.42
Do	do	Stems	—	0
<i>Ficus parvifolia</i> Miq.	Balete	Leaves	—	0
Do	do	Bark	—	0
Do	do	Stems	+	0
<i>Ficus ulmifolia</i> Lam.	Isis	Fruit	—	0
Do	do	Leaves	—	0
Do	do	Stems	+	0.1
<i>Ficus elastica</i> Roxb.	Indian rubber tree	Seeds	—	0
<i>Morus alba</i> Linn.	Amoras	Leaves	—	0
Do	do	Stems	+	0
Do	do	Bark	—	0
<i>Streblus asper</i> Lour.	Kalios	Leaves	+	0.25
Do	do	Stems	—	0
SAPOTACEÆ				
<i>Achras zapota</i> Linn.	Chico	Leaves	—	0
Do	do	Fruit	—	0
<i>Chrysophyllum cainito</i> Linn.	Caimito	Leaves	—	0
Do	do	Stems	—	0
Do	do	Bark	—	0
<i>Madhuca betis</i> (Blanco) Merr.	Betis	Leaves	—	0

TABLE 1.—Results of hemolysis tests and estimated sapogenin contents of plants—Continued.

Species	Local name	Plant part	Hemo- lysis test	Esti- mated total m.f.b.
VERBENACEÆ				Per cent
<i>Avicennia officinalis</i> Linn.	Api-api	Leaves	—	0
Do	do	Stems	+	0.52
<i>Callicarpa formosana</i> Rolfe	Palis	Leaves	—	0
<i>Clerodendron fragrans</i> Vent.	Mil-flores	Leaves	—	0
<i>Clerodendron minahassae</i> Tey. and B.	Bagawak-na-puti	Leaves	—	0
<i>Duranta repens</i> Linn.	Duranta	Leaves	—	0
<i>Premna odorata</i> Blanco	Alagaw	Leaves	—	0
Do	do	Stems	+	0.48
Do	do	Bark	+	0.20
<i>Stachytarpheta jamaicensis</i> (Linn.) Vahl.	Kandi-kandilaan	Leaves	—	0
Do	do	Stems	—	0
<i>Vitex negundo</i> Linn.	Lagundi	Leaves	—	0
Do	do	Stems	+	0.53
<i>Vitex parviflora</i> Juss.	Molave	Leaves	+	0
ZINGIBERACEÆ				
<i>Curcuma longa</i> Linn.	Dilaw	Rhizomes	+	0
Do	do	Leaves	+	0.41
<i>Hedychium coronarium</i> Koenig	Kamia	Leaves	—	0
<i>Zingiber officinale</i> Rosz.	Luya	Rhizomes	—	0
<i>Zingiber zerumbet</i> (Linn.) Sm.	Luiang-usiu	Leaves	—	0
GRAMINEÆ				
<i>Andropogon aciculatus</i> (Retz) Trin.	Amorseco	Leaves	—	0
<i>Andropogon citratus</i> (DC) Stapf.	Tanglad	Leaves	—	0
<i>Andropogon zizanioides</i> (Linn.) Wurm.	Moras	Roots	—	0
Do	do	Leaves	—	0
Do	do	Stems	—	0
<i>Bambusa spinosa</i> Roxb.	Kawayan-tinik	Leaves	—	0
<i>Bambusa vulgaris</i> Schrad.	Kawayang-kiling	Leaves	—	0
<i>Cynodon dactylon</i> (Linn.) Pers.	Kawad-kawaran	Leaves	—	0
<i>Imperata cylindrica</i> (Linn.) Benth.	Kogon	Leaves	—	0
<i>Oryza sativa</i> Linn.	Palai	Leaves	—	0
<i>Saccharum officinarum</i> Linn.	Tabo	Leaves	—	0
Do	do	Stems	—	0
<i>Saccharum spontaneum</i> Linn.	Talahib	Leaves	—	0
<i>Zea mays</i> Linn.	Mais	Seeds	—	0
MELIACEÆ				
<i>Lansium domesticum</i> Correa	Lansones	Fruit	+	0
<i>Melia azedarach</i> Linn.	Paraiso	Stems	—	0
Do	do	Leaves	+	0.65
Do	do	Fruit	—	0
<i>Sandoricum koeljape</i> (Burm. f.) Merr.	Santol	Leaves	—	0
Do	do	Stems	—	0
Do	do	Bark	+	0.20
TILIACEÆ				
<i>Corchorus capsularis</i> Linn.	Pasaw-na-bilog	Leaves	+	0
Do	do	Stems	—	0
<i>Muntingia calabura</i> Linn.	Datiles	Leaves	—	0
Do	do	Stems	—	0
<i>Triumfetta bartramia</i> Linn.	Kulut-kulitan	Leaves	—	0
Do	do	Stems	—	0
ARACEÆ				
<i>Acorus calamus</i> Linn.	Lubigan	Leaves	+	0
<i>Amorphophallus campanulatus</i> (Roxb.) Blume	Pungapung	Stems	—	0
Do	do	Leaves	+	0
<i>Colocasia esculenta</i> (Linn) Schott and Endl.	Gabi	Tubers	—	0
Do	do	Leaves	+	0

TABLE 1.—Results of hemolysis tests and estimated sapogenin contents of plants—Continued.

Species	Local name	Plant part	Hemo- lysis test	Esti- mated total m.f.b.
ANONACEÆ				Per cent
<i>Anona muricata</i> Linn.	Guayabano	Leaves	—	0
Do	do	Stems	—	0
<i>Anona squamosa</i> Linn.	Atis	Bark	—	0
Do	do	Leaves	—	0
Do	do	Stems	—	0
<i>Anona reticulata</i> Linn.	Anonas	Leaves	—	0
Do	do	Stems	+	0.12
<i>Artabotrys suaveolens</i> Blume	Susong-kalabao	Fruit	—	0
<i>Cananga odorata</i> (Linn.) Hooker f. and Thoms.	Ilang-ilang	Flowers	—	0
MYRTACEÆ				
<i>Eucalyptus tereticornis</i> Sm.	Eucalyptus	Leaves	+	0
Do	do	Stems	—	0
<i>Psidium guajava</i> Linn.	Bayabas	Bark	—	0
Do	do	Leaves	—	0
Do	do	Stems	—	0
<i>Syzygium cumini</i> (L) Skeels	Duhat	Leaves	—	0
Do	do	Stems	—	0
Do	do	Bark	—	0
<i>Syzygium malaccense</i> (Linn.) Merr. and Perry	Makopa	Leaves	—	0
Do	do	Stems	—	0
PALMÆ				
<i>Areca catechu</i> Linn.	Bunga	Leaves	—	0
Do	do	Roots	—	0
<i>Arenga pinnata</i> (Wurmb.) Merr.	Kaong	Leaves	—	0
Do	do	Stems	—	0
<i>Cocos nucifera</i> Linn.	Niyog	Bark	—	0
Do	do	Leaves	—	0
Do	do	Stems	+	0
<i>Coryphaea</i> Roxb.	Buri	Leaves	—	0
Do	do	Stems	—	0
<i>Metrozylon sagu</i> Rothb.	Sagu	Trunk	+	0
<i>Nypa fruticans</i> Wurmb.	Nipa	Stems	—	0
Do	do	Leaves	—	0
RUTACEÆ				
<i>Citrus aurantifolia</i> (Christm.) Swingle	Dayap	Fruit	—	0
<i>Citrus aurantium</i> Linn.	Cajel	Fruit	—	0
<i>Citrus grandis</i> Osbeck	Lukban	Leaves	—	0
Do	do	Stems	+	0.28
Do	do	Rinds	+	0
<i>Citrus microcarpa</i> Bunge	Calamansi	Leaves	+	0
Do	do	Stems	+	0
<i>Citrus nobilis</i> Lour.	Darangita	Fruit	+	0.34
<i>Toddalia asiatica</i> (Linn.) Lam.	Dawag	Leaves	—	0
Do	do	Stems	—	0
RUBIACEÆ				
<i>Coffea arabica</i> Linn.	Kafe	Fruit	—	0
<i>Gardenia augusta</i> (Linn.) Merr.	Rosal	Leaves	+	0
Do	do	Stems	—	0
<i>Morinda citrifolia</i> Linn.	Tumbong-aso	Leaves	—	0
<i>Mussaenda philippica</i> A. Rich	Kahoi-dalaga	Leaves	+	0
Do	do	Stems	—	0
<i>Pedderia foetida</i> Linn.	Kantutal	Leaves	—	0
CUCURBITACEÆ				
<i>Benincasa hispida</i> (Thunb.) Cogn.	Kondol	Leaves	+	0
<i>Citrullus vulgaris</i> Schrad.	Pakuan	Fruit	—	0
<i>Cucumis melo</i> Linn.	Melon	Fruit	—	0
<i>Cucurbita maxima</i> Duchesne	Kalabasa	Leaves	—	0
Do	do	Stems	+	0.14

TABLE 1.—Results of hemolysis tests and estimated sapogenin contents of plants—Continued.

Species	Local name	Plant part	Hemo- lysis rest	Esti- mated total m. f. b.
<i>Lagenaria leucantha</i> (Duch.) Rusby..	Upo	Leaves	+	0.13
Do	do	Stems	—	0
<i>Luffa acutangula</i> (Linn.) Roxb.	Patola	Fruit	—	0
<i>Momordica charantia</i> Linn.	Ampalaya	Fruit	—	0
BIXACEÆ				
<i>Bixa orellana</i> Linn.	Achuete	Bark	+	0.13
Do	do	Stems	+	0.64
Do	do	Pulp	+	0
Do	do	Leaves	—	0

TABLE 2.—Wave length positions and intensities of absorption maxima of sulfuric acid chromogens of steroidal sapogenins.

Sapogenin	Absorption Maxima, M μ
Chlorogenin	270,330,415
Diosgenin	271,415,514
Gitogenin	272,308
Hecogenin	276,350,396
Kammogenin	283,272,349
Kryptogenin	280,383
Manogenin	276,348,400,468
Markogenin	270,308
Rockogenin	273,379
Samogenin	270,308
Sarsasapogenin	271,310
Smilagenin	272,312
Tigogenin	270,312
Yuccagenin	240,268,405

SUMMARY

One hundred seventy-two plant samples belonging to seventeen families were analyzed in this investigation. Extracts were prepared from these samples and were subjected to hemolysis test. Crude sapogenin acetates were isolated from fifty-three alcoholic extracts that were positive in the hemolysis test. Substances other than sapogenins which reacted with sulfuric acid were removed by adsorption chromatography, using activated alumina as the adsorbing agent. Acid solution of the sapogenins were determined on a Beckman DU quartz spectropho-

tometer in the ultraviolet region. Twenty-seven samples gave characteristic spectral curves of the steroidal sapogenins. Some of these were the stems of *Avicennia officinalis* Linn., *Bixa orellana* Linn., *Vitex negundo* Linn., the fruit of *Copsicum annuum*. Linn. var. *longum* Sendt., and the leaves of *Melia azedarach* Linn.

Plant samples of the families Tiliaceæ, Sapotaceæ, Palmæ, Gramineæ, and Araceæ were found to be negative.

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MOLECULAR DISTILLATION OF SOME PHILIPPINE VEGETABLE OILS

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Several articles(1, 2, 7) dealing on the molecular distillation of animal and vegetable oils have already been published. Norris Dean Embree's article(5) on the separation of the natural components of fats and oils by molecular distillation, contain data for vegetable oils which show that molecular distillation removes the free fatty acids and the unsaponifiable constituents of the oil with considerable efficiency. This method of concentration is of special value when the substances being studied may be altered by saponification. These substances are Vitamin K, sterol, esters, among others.

Fawcett(6) discussed the application of high vacuum distillation in the processing of triglycerides. He stated that the main advantage of molecular distillation process is its power to distill material at the lowest possible temperature without the need of lengthy exposure of the distillate to that temperature. He summarized the application of molecular distillation to the processing of triglycerides as follows:

- (a) The isolation of the volatile, non-fat constituents such as sterols, vitamins, and others.
- (b) The refining of the triglycerides for edible oil, and others.
- (c) Separation of triglycerides into fractions having properties of special value in specific direction.
- (d) The processing of derivatives of triglycerides such as stand oils, varnishes, and others.

EXPERIMENTAL PROCEDURE

ACMS-5 Centrifugal Cyclic Batch Molecular Still manufactured by Distillation Products, Inc., Rochester 3, New York, was used in the fractional distillation of locally extracted vegetable oils: namely, coconut, raw lumbang, rubber seed, and rice oils. All the oils were distilled at a pressure of 2-1 microns and the fractions were taken at 10°C intervals.

The coconut (*Cocos nucifera*) oil used in this investigation was expressed in a Carver Press from the copra which was available at the time in the laboratory. The data for the molecular distillation of coconut oil are given in Table 1. The fractions have iodine values of from 2.9 to 7.5 and the residue, 43.7, as against the original oil of 9.7, which indicate that some

separation has taken place. This fractionation is also evident in the freezing point of the fractions, ranging from 4.4°C. to 15.1°C., and in the mean molecular weight, which is from 205.0 to 222.6, and the residue, 257.4, while the original oil has only 10.0°C. freezing point and 217.7 mean molecular weight.

Embree(5) stated that similar results were obtained by Bomer and Bauman on coconut oil, using a high vacuum (not a molecular) still. He said further that other workers like Waterman and Rijks distilled coconut oil in an improved high vacuum still and obtained fractions comparable to those shown in Table 1.

TABLE 1.—*Molecular distillation of coconut oil.*

Characteristic	Original oil	Fraction 1	Fraction 2	Fraction 3
Per cent distilled		6.1	27.0	16.2
Distillation temperature, °C		180.0	190.	200.
Iodine number	9.7	6.0	2.9	8.9
Saponification number	257.6	273.1	272.9	266.1
Freezing point °C	10.0	4.4	8.4	9.6
Refractive index ⁿ _D 37 °C	1.4480	1.4454	1.4470	1.4479
Mean molecular weights	217.7	205.0	205.5	210.8
Free fatty acids (as oleic) per cent	1.8	13.7	5.3	1.0
Melting point, °C	24.9	18.4	22.9	25.3

Characteristic	Fraction 4	Fraction 5	Residue
Per cent distilled	12.8	28.8	10.6
Distillation temperature, °C	210.0	220.0	
Iodine number	4.9	7.5	43.7
Saponification number	261.9	252.0	217.9
Freezing point °C	12.1	15.1	12.6
Refractive index ⁿ _D 37 °C	1.4472	1.4490	1.4555
Mean molecular weights	214.2	222.6	257.4
Free fatty acids (as oleic) per cent	0.6	0.3	1.4
Melting point, °C	27.5	28.8	28.0

The lumbang oil used in this research was kindly presented to us by Elizalde Oil Factory, Manila, Philippines, and was prepared from the nuts of *Aleurites moluccana*, grown in the Philippines. This oil is a drying oil which is used in making paints, varnishes, and similar products. Cruz and West(3) investigated the composition of this oil and their results indicate that the oil consists principally of the glycerides of linolenic, oleic and various linolic acids. The data in Table 2 give some of the properties of the fractions from the molecular distillation of Philippine lumbang (candlenut) oil. The iodine values vary only from 139.3 to 157.5, which show that there is hardly any separation of the triglycerides in the oil. The original oil has an iodine value of 156.1.

TABLE 2.—Molecular distillation of lumbang oil.

Characteristic	Original oil	Fraction 1	Fraction 2	Fraction 3
Per cent yield		2.7	8.9	13.6
Distillation temperature, °C		130-140	190-210	220
Iodine number	156.1	139.3	137.1	149.6
Saponification number	194.3	195.6	199.1	195.6
Refractive index— $\frac{n}{D}$ 37 °C	1.4685	1.4635	1.4682	1.4689
Mean molecular weights	288.7	286.8	281.8	286.8
Free fatty acids (as oleic), per cent	2.5	73.9	2.3	0.3

Characteristic	Fraction 4	Fraction 5	Fraction 6	Residue
Per cent yield	15.9	.9	20.4	10.00
Distillation temperature, °C	230-240	250-260	270.0	
Iodine number	152.2	153.9	157.5	154.0
Saponification number	194.9	193.6	191.9	203.5
Refractive index— $\frac{n}{D}$ 37 °C	1.4693	1.4700	1.4705	1.4753
Mean molecular weights	287.8	289.8	292.2	275.7
Free fatty acids (as oleic), per cent	0.1	0.1	0.1	1.0

The para-rubber (*Hevea brasiliensis*) seed oil used in this work was prepared from para-rubber seeds which were kindly given to us by Dr. Enrique Virata of the University of the Philippines. The seeds were obtained from plants grown in the Philippines. Tuason and Cruz(8) published recently their work on the composition of para-rubber seed oil. This oil, which belongs to the same class as Philippine lumbang oil, possesses drying properties. The percentage of saturated glycerides is however quite high, 14.6 per cent. In Table 3, are given the analyses of fractions obtained in the molecular distillation of para-rubber seed oil. The data (Table 3) show that the iodine values of the fractions are from 134.4 to 136.0, while the original oil has 136.7 iodine value. The results indicate that there was no separation of the saturated glycerides from the unsaturated glycerides by molecular distillation of the oil.

TABLE 3.—Molecular distillation of rubber seed oil.

Characteristic	Original oil	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Per cent yield		9.01	6.01	4.3	5.2
Distillation temperature, °C		140.0	150.0	190.0	200-210
Iodine number	136.7	134.4	133.6	115.0	122.5
Saponification number	196.7	216.9	200.7	197.5	192.3
Refractive number— $\frac{n}{D}$ 37 °C	1.4669	1.4660	1.4645	1.4701	1.4692
Mean molecular weights	285.2	258.6	279.5	284.1	291.7
Free fatty acid (as oleic) per cent	11.70	98.89	93.35	16.26	2.05

TABLE 3.—Molecular distillation of rubber seed oil—Continued.

Characteristic	Fraction 5	Fraction 6	Fraction 7	Residue
Per cent yield.....	9.6	9.7	16.0	37.9
Distillation temperature, °C.....	220-230	240.0	250.0	-----
Iodine number.....	126.8	129.4	181.4	186.0
Saponification number.....	195.2	196.5	195.7	199.3
Refractive index— n_D^{37} °C.....	1.4720	1.4708	1.4686	1.4699
Mean molecular weights.....	287.4	285.5	286.7	281.5
Free fatty acid (as oleic) per cent.....	1.15	0.65	0.35	0.82

The rice (*Oryza sativa*) oil used in this experiment was obtained by extraction with ether from Philippine rice bran. Cruz and West(4) investigated recently Philippine rice oil and their results showed that the oil consists principally of glycerides of oleic, linolic, and palmitic acids. In Table 4 are given the physical and chemical constants of the fractions, which were obtained by molecular distillation of the oil. Since the original rice oil had an iodine number of 96.1 and the fractions had iodine values ranging from 86.9 to 102.3, it is apparent that some separation of the glycerides occurred.

TABLE 4.—Molecular distillation of rice bran oil.

Characteristic	Original oil	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Per cent yield.....	-----	12.1	11.1	8.8	6.3
Distillation temperature, °C.....	-----	100.0	110.0	120.0	130.0
Iodine number.....	96.1	86.9	99.1	86.7	161.9
Saponification number.....	190.5	184.1	200.9	161.8	182.9
Freezing point °C.....	3.4	33.4	22.1	6.2	2.4
Refractive index— n_D^{37} °C.....	1.4650	1.4518	1.4580	1.4735	1.4680
Mean molecular weights.....	294.5	304.7	279.2	346.7	306.7
Unsaponifiable matter, per cent.....	5.3	7.3	7.4	8.1	7.3
Free fatty acid (as oleic) per cent.....	22.5	90.5	74.3	17.7	3.0

Characteristic	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Residue
Per cent yield.....	9.2	4.7	11.2	22.0	9.1
Distillation temperature, °C.....	140.0	150-160	165-175	180.0	-----
Iodine number.....	100.9	96.1	97.2	101.4	102.29
Saponification number.....	185.3	190.6	187.5	201.6	189.3
Freezing point °C.....	below-2	below-2	below-2	below-2	3.0
Refractive index— n_D^{37} °C.....	1.4669	1.4655	1.4644	1.4625	1.4775
Mean molecular weights.....	302.7	294.3	299.2	278.2	296.3
Unsaponifiable matter, per cent.....	6.8	4.0	2.9	2.6	5.9
Free fatty acid (as oleic) per cent.....	1.8	1.4	1.0	0.4	2.3

All the four Philippine vegetable oils gave very high percentages of free fatty acids in the first and second fractions. These first fractions were highly odoriferous. The unsaponi-

fiable matter was concentrated in these first fractions. All the fractions were pale in color, while the residues were highly colored.

In the coconut and rice oils, the fractions showed differences in iodine values and mean molecular weights, which indicate that some fractionation of their glycerides took place. However the drying properties of lumbang and rubber seed oils were not improved by molecular distillation.

SUMMARY

Four Philippine vegetable oils, namely, coconut, lumbang, rubber seed, and rice oils were fractionally distilled in a CMS-5 Centrifugal Cyclic Batch Molecular Still.

The first and second fractions in all the oils, were highly odoriferous. These fractions were high in free fatty acids content and rich in unsaponifiable matter.

All the fractions were light in color, while the residues were highly colored.

Some separation of the glycerides occurred as shown in the iodine values of the fractions in the case of coconut and rice oils.

Lumbang and rubber seed oils did not improve in their drying properties when molecularly distilled as shown in the iodine numbers of the different fractions.

The above observations are similar to those reported by other workers.

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THE COMPOSITION OF DILIS FISH FLOUR¹

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Dietary lack of high quality protein from animal sources is one of the nutritional problems in the Philippines, where a large number of people of limited means obtain their caloric requirements chiefly from rice and other cereals.

Fish, which is a common item in the Filipino diet, is a cheap source of animal protein. *Dilis* or "long-jawed anchovy" (*Stolephorus Commersonii*) is one such fish in the Philippines that is available to all people of whatever income. Sometimes made into a fish paste called bagoong, the raw fish is more commonly salted and dried and is either toasted or fried for food. Eaten whole with the bones, the fish may be an important source of calcium. Leverton and Payawal,⁽¹⁶⁾ using a group of 9- to 13-year-old girls, studied the physiological availability of calcium, phosphorus and nitrogen from the bones and flesh of dilis. They found that the girls could utilize the calcium, phosphorus and nitrogen from the flesh and bones of dilis as well as they could utilize the same from milk. This finding has stimulated interest in the possibilities of dilis as a source of a cheap and yet good quality protein.

Dilis fish flour has been made on a small scale by the Philippine Institute of Fisheries Technology to serve as a supplement in improving the quality of protein of the average Filipino diet. Of the two forms made by the Institute, raw fish flour is prepared by washing the fresh dilis in water and then drying it thoroughly under the sun or in the air. The dried fish is then passed through a ball mill until finely powdered. The deodorized fish flour, the other form, is prepared by extracting the raw fish flour three or four times with alcohol and then drying the extract at low temperature by evaporation. The resulting product is nearly odorless, tasteless, and saltless; it is more acceptable to many people than the raw flour.

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The present study was undertaken to determine the proximate analysis, amino acid and vitamin content of these two flours. Sixteen amino acids, including the eight essential ones, niacin, pantothenic acid and vitamin B₁₂ have been determined microbiologically.

PROCEDURE

In the proximate analysis of the flours the methods recommended by the Association of Official Agricultural Chemist(1) were followed.

The microbiological methods used are based on the principle that certain lactic acid producing bacteria require amino acids and vitamins plus other nutrients for growth. This growth is measured quantitatively by titrating the amount of lactic acid produced with a standard alkali. If the bacteria are grown in a medium adequate to meet the requirements of the organism, but deficient of the amino acid or vitamin being determined, the addition of the substance being quantitated will produce growth directly proportional to the amount of the particular amino acid or vitamin added.

Table 1 shows the organisms employed and the methods used in the microbiological assay of the sixteen amino acids, niacin, pantothenic acid and vitamin B₁₂ in dilis fish flour. The Appendix shows in detail the methods employed and the modifications used which gave the best results in the study.

TABLE 1.—Methods for microbiological assays.

Nutrient	Organism	Method ¹
Amino acids		
L-Isoleucine.....	<i>Leuconostoc mesenteroides</i> P-60..	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Leucine.....	<i>Leuconostoc mesenteroides</i> P-60..	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Lysine.....	<i>Leuconostoc mesenteroides</i> P-60..	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Tryptophan.....	<i>Leuconostoc mesenteroides</i> P-60..	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Aspartic acid.....	<i>Leuconostoc mesenteroides</i> P-60..	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Histidine.....	<i>Leuconostoc mesenteroides</i> P-60..	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)

TABLE 1.—Method for microbiological assays—Continued.

Nutrient	Organism	Method ¹
Glycine-----	<i>Leuconostoc mesenteroides</i> P-60--	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Proline-----	<i>Leuconostoc mesenteroides</i> P-60--	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Serine-----	<i>Leuconostoc mesenteroides</i> P-60--	Henderson and Snell (11), Chitre, et al. (10), Violante, et al. (25) and Steele, et al. (22).
L-Methionine-----	<i>Leuconostoc citrovorum</i> 8081-----	Henderson and Snell (11), Chitre, et al., (10) Violante, et al., (25)
L-Tyrosine-----	<i>Leuconostoc citrovorum</i> 8081-----	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Threonine-----	<i>Streptococcus faecalis</i> -R ⁺ -----	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Phenylalanine-----	<i>Lactobacillus arabinosus</i> 17-5-----	Henderson and Snell (11), Chitre, et al. (10), and Violante, et al. (25)
L-Glutamic acid-----	<i>Lactobacillus arabinosus</i> 17-5-----	Henderson and Snell (11), Chitre, et al., (10) and Violante, et al., (25) and Brickson, et al. (8)
L-Valine-----	<i>Lactobacillus delbrueckii</i> 3-----	Henderson and Snell (11), Chitre, et al., (10) Violante, et al., (25) and Meinke and Holland (19)
L-Arginine-----	<i>Lactobacillus delbrueckii</i> 3-----	Henderson and Snell (11), Chitre, et al., (10) Violante, et al., (25) and Meinke and Holland (19)
Niacin-----	<i>Lactobacillus arabinosus</i> 17-5-----	Association of Official Agricultural Chemists (2)
Pantothenic acid-----	<i>Lactobacillus arabinosus</i> 17-5-----	Association of Official Agricultural Chemists (2) and Ives and Strong (12)
Vitamin B ₁₂ -----	<i>Lactobacillus leichmannii</i> -----	Pharmacopoeia of the United States of America, (26) Berman et al., (6) and Schweigert, et al. (21)

¹ Numbers in parentheses refer to references.

The assays were carried out in 18 × 150 mm pyrex culture tubes. The tubes were held in copper racks of 60 tubes per rack. Standards and hydrolyzed samples at five dilutions of 0.2, 0.4, 0.6, 0.8, and 1.0 ml were prepared in duplicate. Also prepared in duplicate with each rack were similar dilutions of a hydrolyzed "recovery mix" and a hydrolyzed casein solution for the purpose of determining recoveries. The "recovery mix" was prepared by hydrolyzing together equal amounts of casein and the dilis flour being tested. Each dilution was made up

to a volume of 1 ml by the addition of distilled water. One milliliter of medium was added to each tube, making a total volume of 2 ml. A copper cover, three inches deep and lined with a thick padding of cotton between gauze, was placed over each rack. The tubes were sterilized by autoclaving for 5 minutes at 15 pounds pressure. After cooling, a drop of a suspension in 0.9 per cent KCL solution of a freshly washed 15-hour culture of the assay organism was added to each tube from a sterile syringe. The tubes were incubated from 64 to 72 hours at 37°C. The lactic acid produced was titrated with 0.05 N KOH. An automatic dispenser and titrator was used in the dispensing and titration processes involved in the assay. The quantity of the amino acids or vitamins in the samples was determined by comparing the volume of the alkali required to titrate the samples with that required to titrate the standard. To check the validity of the results, the per cent recoveries were determined from the values obtained from the recovery mix and the casein hydrolysate. Only results that showed a recovery of 90 to 110 per cent were included and those results outside this range were discarded.

For the determination of the different amino acids except tryptophan, acid hydrolysates of the samples in duplicate were prepared. The samples were hydrolyzed in covered pyrex Erlenmeyer flasks by autoclaving for ten hours at 15 pounds pressure. Forty milliliters of 3 N HCL per gram of sample was used for the hydrolysis. The acid hydrolysates were adjusted to pH 5, filtered and then diluted to 100 ml.

For the determination of tryptophan, alkali hydrolysates of the samples were prepared in duplicate by autoclaving for 24 hours at 15 pounds pressure. Twenty milliliters of 5 N KOH per gram of sample were used to hydrolyze the sample in stainless steel beakers. Alkali hydrolysates were then filtered and diluted to 100 ml.

Both acid and alkali hydrolysates were stored under toluene and refrigerated until needed. Aliquot portions of the hydrolysates were first adjusted to pH 6.8 before being used in the assay.

The composition of the medium used was that recommended by Henderson and Snell,⁽¹¹⁾ with some modifications as suggested by Brickson, et al.,⁽⁸⁾ Meinke and Holland⁽¹⁹⁾ and

by Steele, et al.(22) Sodium acetate was used instead of potassium acetate in the medium as the organism employed showed requirement for sodium.

To eliminate variations due to possible salt effects as reported by Camien, et al.,(9) the salt concentration of the amino acid standard was adjusted to the concentration of the salt in the neutralized hydrolysate by the addition of potassium chloride, following the suggestions of Chitre, et al.(10) and Violante, et al.(25)

The hydrolyzed sample for the determination of niacin was prepared by autoclaving 0.5 gm of sample with 5 ml 1.0 N H_2SO_4 at 15 pounds pressure for 30 minutes. The hydrolysate was cooled and adjusted to pH 6.8 with 1.0 N KOH. It was then filtered and diluted to 100 ml with distilled water, stored under toluene and refrigerated. Standard of niacin with a concentration of 0.2 mcg per ml used in the assay.

For the determination of free pantothenic acid, an enzyme hydrolysate of the sample was prepared following the method of Ives and Strong.(12) Five-tenths gram of sample was suspended in 2.5 molar sodium acetate solution. This mixture was brought to a pH of 4.8 with 0.5 N HCl and 50 mg of the enzyme, mylase, was added. The mixture was incubated for approximately 20 hours at 37°C., and the pH was then again adjusted to 4.8 diluted to 100 ml and filtered. A dilution of 1 to 500 of the sample was used in the assay. The standard pantothenic acid solution had a concentration of 0.04 mcg per ml.

Samples for the vitamin B_{12} assay were prepared by autoclaving 0.5 gm of the sample with 25 ml of a buffer solution of sodium acetate, as suggested by Berman, et al.,(6) for 20 minutes at 15 pounds pressure. After cooling, the sample was brought to PH 6.0, filtered and made up to a volume of 100 ml. Standard vitamin B_{12} solution with a concentration of .5 mcg per ml was used.

RESULTS AND CONCLUSIONS

Table 2 gives the results of the proximate analysis of the dilis fish flours together with the figures for other fish flours reported in literature.

TABLE 2.—Proximate composition of fish flours.

Constituents	Dilis fish flour		Fish meal (17) ¹		Perch (14) ¹	Shad (15) ¹	Had-dock (15) ¹	Pom-fret (15) ¹
	Raw	Deo-dorized	Raw	Deo-dorized				
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Nitrogen.....	12.66	12.27	12.84	12.73	12.60	14.16	14.4	13.4
Protein (N×6.25).....	79.12	76.68	80.30	79.60	78.70	88.56	90.31	84.37
Fat.....	0.0	0.0	0.08	trace	7.83	0.40	0.44	0.62
Moisture.....	5.39	9.33	1.87	5.5	7.50	6.67	7.91	8.40
Ash.....	13.64	13.19	14.5	15.3	6.60	5.40	4.24	8.05

¹ Number refers to references.

Values of raw fish flour do not differ significantly from those of deodorized dilis flour. Nitrogen content of dilis flours is similar to that of fish meals as reported by Levin(17) and of perch meal as reported by Kik,(14) but is lower than those of shad, hadlock and pomfret as reported by Lahiry and Proctor.(15) Dilis flours and fish meals(17) have higher values for ash than the shad, hadlock, pomfret and perch. This is probably due to the inclusion of the bones in the preparation of dilis fish flours and fish meals.(17) In the preparation of flours from shad, hadlock, pomfret and perch only the fish muscle was used.

Determination of the calcium content of dilis flours in this study showed that the raw flour had 3.92 gm and the deodorized flour had 3.94 gm calcium per 100 gm of air-dried flour.

In Table 3, the amino acid composition of raw and deodorized dilis flours is given in grams per hundred grams of air dry sample and also in terms of per cent of crude protein, that is,

TABLE 3.—Amino acid composition of dilis flour.

Amino acid	Air dry dilis flour		Calculated to 16.0 gm. (N)	
	Raw ¹	Deodorized ²	Raw	Deodorized
	gm/100 gm	gm/100 gm	gm	gm
L-Isoleucine.....	4.0	4.6	5.1	5.3
L-Leucine.....	6.1	6.3	7.7	8.2
L-Lysine.....	7.3	7.4	9.3	9.7
L-Methionine.....	2.8	2.9	3.6	3.7
L-Phenylalanine.....	3.4	3.6	4.3	4.7
L-Threonine.....	3.7	3.7	4.6	4.8
L-Tryptophan.....	0.9	1.0	1.1	1.3
L-Valine.....	4.5	4.3	5.7	5.9
L-Arginine.....	3.9	4.0	4.9	5.3
L-Aspartic acid.....	7.5	8.6	9.4	11.2
L-Glutamic acid.....	9.3	9.0	11.8	11.7
Glycine.....	4.3	3.7	5.4	4.8
L-Histidine.....	2.5	2.8	3.1	3.6
L-Proline.....	3.2	3.0	4.0	3.9
L-Serine.....	3.8	3.9	4.8	4.9
L-Tyrosine.....	3.2	3.4	4.0	4.4

¹ 5.39 per cent moisture, 13.64 per cent ash, 12.66 per cent nitrogen.² 9.33 per cent moisture, 13.19 per cent ash, 12.27 per cent nitrogen.

grams of amino acid per 16 grams total nitrogen. Values of deodorized flour are similar to those of raw flour indicating that deodorization did not affect significantly the amino acid content of the raw dilis flour.

For purposes of comparison, amino acid composition of deodorized dilis flour with values reported in literature for a variety of protein foods including other fish meals are presented in Table 4. This table reveals a striking similarity in the tryptophan content of deodorized dilis flour with casein, whole egg, beef muscle, perch and other fish meals. Deodorized

TABLE 4.—*Comparison of amino acid composition of deodorized dilis flour and certain other animal protein foods.*

[Calculated as grams per 16.0 grams nitrogen]

Amino acid	Deodorized dilis flour	Casein (7) ¹	Whole egg (7) ¹	Beef muscle (20) ¹	Perch (14) ¹	Shad muscle (15) ¹	Fish flakes (20) ¹	Fish muscle (7) ¹	Bombay fish varieties (18) ¹
L-Isoleucine.....	5.3	7.5	7.7	5.0	5.0	5.0	5.5	6.5	3.4-7.3
L-Leucine.....	8.2	10.0	9.2	7.8	7.1	7.7	8.1	9.5	6.2-14.3
L-Lysine.....	9.7	8.5	7.0	8.7	8.1	9.8	8.8	9.0	16.5-19.3
L-Methionine.....	3.7	3.5	4.0	2.7	4.8	3.7	3.0	3.2	1.6-4.6
L-Phenylalanine.....	4.7	6.3	6.3	3.8	4.6	2.8	3.9	4.4	2.1-4.6
L-Threonine.....	4.8	4.5	4.3	4.5	4.8	4.0	4.9	4.7	4.108.1
L-Tryptophan.....	1.3	1.3	1.5	1.0	1.3	1.1	1.0	1.2	0.2-1.0
L-Valine.....	5.9	7.7	7.2	5.2	6.2	6.1	5.3	6.0	2.9-6.5
L-Arginine.....	5.3	4.2	4.2	6.1	7.3	5.7	6.1	7.4	3.7-8.3
L-Aspartic acid.....	11.2	7.0	-----	-----	6.4	-----	-----	-----	-----
L-Glutamic acid.....	11.7	23.0	-----	-----	14.6	-----	-----	-----	-----
Glycine.....	4.8	2.1	2.7	-----	6.5	-----	-----	-----	-----
L-Histidine.....	3.6	3.2	2.4	3.6	2.2	2.3	2.1	2.6	1.2-1.9
L-Proline.....	3.9	13.1	-----	-----	3.0	-----	-----	3.0	-----
L-Serine.....	4.9	6.8	-----	-----	6.4	-----	-----	4.0	-----
L-Tyrosine.....	4.4	6.4	4.5	-----	3.8	-----	-----	3.8	0.8-1.6

¹ Number refers to references.

fish flour has a higher lysine and threonine content than casein, whole egg, and beef muscle, and a slightly greater histidine content than casein and whole egg. Values of other essential amino acids of dilis flour do not differ greatly from the values reported for casein, whole egg and beef muscle. Of the non-essential amino acid content, deodorized dilis flour has a higher aspartic acid, arginine and glycine content than casein, but has a lower proline, serine and glutamic acid content than casein. When compared with the other fish meals, deodorized dilis flour compares closely with those reported by Kik(14) for perch, Lahiry and Proctor(15) for shad muscle, Niелands, et al.,(20) for fish flakes, Block and Bolling(7) for fish muscle, and Master and Magar(18) for eight varieties of Bombay fishes.

One concept that has emerged from recent nutrition research is the importance of the proportions of the different amino acids in food. This means that the nutritional value of any

diet is dependent in part on the ratios existing among the amino acids present. Albanese, et al.,⁽³⁾ have reported that the nitrogen retention of underweight infants could be enhanced by lysine supplementation to bring their milk diets to the lysine tryptophan ration (L:T) of meat. According to these authors, an L:T ratio in the range of 5 to 7 is associated with the highest efficiency of protein utilization. Dilis fish flours have an L:T ratio of 7.4.

In Table 5 the niacin, free pantothenic acid and vitamin B₁₂ content of both raw and deodorized dilis flours are shown, together with the results on perch reported by Kik.⁽¹⁴⁾ Deo-

TABLE 5.—*Vitamin content of fish flour.*

Vitamin	Raw dilis flour	Deodorized dilis flour	Perch (14)
	<i>per 100 gm.</i>	<i>per 100 gm.</i>	<i>per 100 gm.</i>
Niacin, mg -----	7.1	3.5	5.1
Pantothenic acid, mg -----	0.4	0.2	1.0
Vitamin B ₁₂ , mcg -----	17.9	15.8	-----

dorized fish flour has lower values than raw fish flour for the three vitamins tested. The niacin content of dilis flours is close to that of perch but their free pantothenic acid content is lower. The vitamin B₁₂ content of dilis flours is within the range of values for vitamin reported in the literature. Tarr, et al.,⁽²⁴⁾ reported the vitamin B₁₂ content of fish products such as fish organs and flesh to be 7.2 to 18 mcg per 100 gm of material. Kamisaka, et al.,⁽¹³⁾ reported the vitamin B₁₂ content of Japanese commercial fish meal to be 8 to 16 mcg per 100 gm. Raw dilis flour and deodorized dilis flour have 17.9 mcg and 15.8 mcg vitamin B₁₂, respectively, per 100 gm air dry flour.

Bender and Haizelden⁽⁵⁾ studied the biological value of the proteins of 27 fish meals and deodorized fish flours for rats by determining the net protein utilization and digestability by carcass analysis. They reported a range from 18 to 80 per cent of net protein utilization. They also found no consistent difference in the results between the defatted and the deodorized flour made from the same kind of fish. Sure⁽²³⁾ reported that an increase in the body weight and higher protein efficiency ratio can be obtained in the albino rat by the addition of small amounts of defatted fish flour to millet and sorghum diets. Kik⁽¹⁴⁾ studied the growth and metabolism of young rats fed on diets supplemented with perch meal. He found that when

1, 3, or 5 per cent perch replaced an equivalent amount of protein in milled rice, an increase in body weight of 111.1, 111.1 and 100 per cent respectively were obtained.

Autret and van Veen(4) suggested the use of fish flour as a possible source of proteins in child feeding. They believe that edible fish flour offers a cheap and yet convenient source of proteins for the child because of its high content of good quality protein. In addition, fish flour is a good source of calcium and vitamin B₁₂.

Because rice is deficient in lysine and threonine, dilis flour may be used to improve the quality of protein in diets where this staple predominates. It has the advantage of having good keeping quality and portability. In powdered form, it has less bulk and can be made available to areas far from sources of fresh fish or fresh animal protein. It easily blends with milk, soups, cakes, cookies and other food preparations.

The infant, especially after weaning, the growing child, the adolescent, the lactating mother and other persons who need increased protein could be benefited by the addition of dilis fish flour to their diets.

SUMMARY

The proximate analysis, amino acid composition, calcium, niacin, pantothenic acid and vitamin B₁₂ content of raw and deodorized dilis fish flours were determined. Results show that dilis fish flour has a protein content of 76.68 to 79.12 per cent and an ash content of 13.19 to 13.64 per cent and is practically fat free. The calcium content is 3.92 and 3.92 gm per 100 gm of the air dry raw and deodorized flours respectively. The essential amino acid composition of dilis fish flour compares closely to values reported in literature for casein, whole egg, beef muscle and other varieties of fish. Results also show that dilis fish flour is an excellent source of lysine and threonine, in which rice, the mainstay of the Filipino diet, is deficient. The niacin and vitamin B₁₂ content of dilis flour is similar to the values reported in literature from other fish sources.

Dilis fish flour could be of great value in supplementing diet in which rice and vegetable predominate by improving the quality of the protein and also in serving as an additional source of calcium and vitamin B₁₂.

ACKNOWLEDGMENT

This work was done at the Oklahoma State University at Stillwater where the senior author held an Ida H. Hyde Inter-

national Fellowship in 1956-1957 granted her through the American Association of University Women. Her special thanks go to Mrs. Florence S. Waddill, Mr. George Odell, Mrs. Ruby Moore, Miss Gwen Slover, Dr. Dorothy Steel, Miss Esther Winterfeldt, and Mrs. Valencia Stone, all of this University, for many forms of assistance they rendered her.

The fish flours used in the study were generously provided for by the Philippine Institute of Fisheries Technology, Manila.

APPENDIX

I. Medium for the microbiological assay of amino acids.

The following modifications on the basal medium suggested by Henderson and Snell⁽¹¹⁾ were made:

1. Folinic acid solution was added to the medium whenever the assay organism used was *Leuconostoc citrovorum* 8081. According to Steele, et al.,⁽²²⁾ *Leuconostoc citrovorum* 8081 has a requirement for folinic acid or a substance found in liver concentrate. A stock solution containing 5 mcg per ml of folinic acid was prepared and kept refrigerated until needed. Two milliliters of this solution were added for every 100 ml of medium used in the assay.
2. Calcium chloride solution was added to the medium when the assay organism used was *Lactobacillus delbrueckii* 3. Meinke and Holland⁽¹⁹⁾ reported that the organism has a calcium requirement. Five grams $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ were dissolved in 100 ml distilled water as a stock solution. For each 100 ml of media, 0.4 ml calcium chloride solution was added.
3. In the assay for glutamic acid, asparagine was used in place of aspartic acid. According to Brickson, et al.,⁽⁸⁾ there exists an antagonism between glutamic and aspartic acid if both are present in the media. Instead of aspartic acid solution, dry asparagine in a concentration of one-half the amount of aspartic acid was added to the media after the media was adjusted to pH 6.0.

- ##### II. Preparation of the sample solution for the assay of free pantothenic acid.
- Instead of the AOAC method⁽²⁾ for preparing the sample, the modification of Ives and Strong⁽¹²⁾ was followed. One-half gram of fish flour sample was weighed on an analytical balance and then suspended in 25 ml distilled water. The suspension was brought to a pH of 6.8, autoclaved for 15 minutes at 121°C., cooled, and 1 ml of 2.5 molar sodium acetate solution was added. The solution was adjusted to pH 4.8 with 0.5 N HCl and 50 mg of the enzyme, mylase, was added. This mixture was incubated for 20 hours at 37°C., after which the pH was again adjusted to 4.8, and then the volume was made up to 100 ml. The sample was filtered and stored under toluene and kept refrigerated until needed. Twenty milliliters of filtrate were made up to 50 ml for use at each assay.

III. For the vitamin B₁₂ assay, the medium recommended by the U.S.P.

XV was modified by the use of an amino acid mixture. The amino acid mixture was used because the casein hydrolysate called for in the U.S.P. method gave poor growth.

Composition of amino acid mixture:

	Gm
L-Glutamic Acid	1.6
L-Asparagine	1.6
L-Lysine	0.8
L-Cysteine-HCl	1.6
L-Leucine	0.4
DL-Threonine	0.8
DL-Valine	0.8
DL-Isoleucine	0.8
DL-Alanine	0.8
DL-Methionine	0.6
L-Arginine-HCl	0.4
L-Histidine × HCl	0.4
DL-Tryptophan	0.8
L-Tyrosine	0.4
DL-Serine	0.4
DL-Phenylalanine	0.4
L-Proline	0.2
Glycine	0.4

Add HCl to bring into solution. Make to 250 ml with distilled water. Ten milliliters of this solution per 100 ml of medium were used.

A one-hour casein hydrolysate was also added to the medium as a source of stimulatory peptides. To prepare this hydrolysate, 1 gm of vitamin-free casein was hydrolyzed with 3.0 ml of 3 N HCl by autoclaving at 121°C. for 1 hour. The hydrolysate was immediately adjusted to pH 7.0 after removal from the autoclave and then made to 100 ml volume and filtered. Ten milliliters of this solution were added per 100 ml of medium.

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BIOLOGICAL QUALITY OF DILIS FISH FLOUR USED AS SOURCE OF PROTEIN FOR GROWING RATS¹

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ONE PLATE

Lack of good quality proteins in the diet is the chief nutritional problem in many areas of the world. Meats, milk, and eggs are expensive and are often beyond the reach of the average family. Increased individual productive capacity and resistance to infection of workers can only be attained with proper nutrition. This is not easy to do especially in large population areas of limited means.

The role that proteins play in the building and repair of body tissues and their relation to the formation of antibodies are well known. Although the causes of the disease Kwashiorkor have not yet been definitely established, there are strong indications that it is due to protein malnutrition. The Food and Agricultural Organization of the United Nations and individual government agencies are now engaged in looking for an inexpensive supply of good quality protein to remedy existing problems.

The Philippines is a rice-eating country where most of the caloric requirement of the average family is supplied by rice. According to Quiogue,(8) previous dietary surveys on 559 families of laborers in rural parts of the Philippines showed the following caloric distribution: cereals, mainly rice, 83.4 per cent; animal protein foods, 6.6 per cent; milk and dairy products, 0.1 per cent; sugar, candy, and fats, 2 per cent; and fruits, vegetables and legumes, 7.8 per cent. Typical diets in rural areas are therefore likely to be deficient in animal protein, fat, calcium, vitamin C, and the vitamin B complex. Burch, et al.,(3) reported

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serum protein levels are in the lower range of normal among the subjects studied in Bataan Province, Philippines.

Autret, et al.,⁽²⁾ of the Nutrition Division of FAO, reported that preliminary results of feeding experiments in Dakar showed that fish flour can be given to infants as early as the third or fourth month provided the fat content of the flour is not higher than 3 per cent. When the fat content reaches 5 per cent the fish flour can still be tolerated by infants more than 6 months old and at a 7 to 8 per cent fat content by children one to two years old.

Kik⁽⁴⁾ reported that the proteins of perch had a supplementary value to those of milled rice when added in levels of 1 per cent, 3 per cent, and 5 per cent. In his work on the nutritional improvement of rice he studied the growth response of young rats on a diet of milled rice supplemented with dried fish meal prepared from perch filets.

Richey, et al.,⁽⁹⁾ found that supplementing a soy protein type of purified diet for chicks with a 10 per cent fish meal uniformly gave a marked growth response.

In the Philippines, interest on the possibilities of dilis fish as a cheap but good source of necessary food nutrients was stimulated by the findings of Leverton and Payawal⁽⁵⁾ who studied the physiological availability of calcium and phosphorus from dilis on a group of 9- to 13-year-old children. They found that girls of this age could utilize the calcium, phosphorus, and nitrogen from the bones and flesh of dilis as well as they could those from milk.

A. T. Suaco and Company, a pharmaceutical manufacturing company of high repute in Manila, Philippines, has recently put on the market a protein hydrolysate of this native fish under the trade name "Dilex" tablets, for the prevention and treatment of certain conditions requiring high protein therapy.

Anchovy, locally called *dilis* (*Stolephorus Commersonii*) of the family Engraulidæ is the best fish for making fish flour in the Philippines. This fish, which is abundantly found in Philippine waters throughout the year, is a small flat fish about 2 to 3 inches long with soft scales and bones. It is usually eaten whole in dried or fried form. According to Villadolid and Pidlaogan,⁽¹¹⁾ the raw fish flour is prepared by thoroughly washing the fresh dilis in water and drying it under the sun or air. Commercially dried salted dilis, if used, is first dipped in water, is thoroughly washed to remove the salts, and is dried. The dried fish is then

pulverized by passing through a mechanical grinder. To remove the fishy odor the raw fish flour is washed three or four times by repeated extraction with hot refined ethyl alcohol (60 to 70°). The residue is dried in the sun or in an oven at 80°C. until all the solvent is evaporated. The resulting product is nearly odorless, tasteless, saltless, and entirely edible.

The purpose of the present study was to determine the biological quality and food efficiency of both raw and deodorized dilis flours. Cheapness, wide distribution, and availability of this small fish throughout the year in the Philippines, which would make it possible to bring a good quality protein within the reach of everyone, make this study of practical importance.

PROCEDURE

Animals used.—Forty-five weanling female, albino rats of the Sprague-Dawley strain with initial weights of 36.0 to 48.2 gms were used for the study. The rats, which were left unfed for 12 hours, were grouped into three rats of similar weight in each group.

Nine groups of three rats each were used for growth studies. Each group was placed in an animal cage of the army medical school type.

For the metabolism studies, eighteen rats were placed in individual metabolism cages 11 inches high and 9 inches wide. They were placed on a 10-inch pyrex pie plate which contained 4 ml of 3 N HCl. Each plate was covered with a fine-mesh screen which served to collect the feces and to allow the urine to drop through into the plate. The top of each cage was closed with a thin 11-inch pie tin plate held securely by two spring wires. The feeders were made of galvanized iron suspended from the upper edge of the cages. A 50 ml beaker was used as the feed container. It was held in place by a cup which was soldered to the floor of the feeder. This apparatus satisfactorily prevented the rats from scattering food.

The work was carried out in an air-conditioned room whose temperature was maintained at $27 \pm 2^\circ\text{C}$. for the 45-day experimental period.

Diets prepared.—Nine diets were prepared, three containing casein as the protein source, three, raw dilis flour, and three, deodorized dilis flour. The amount of casein and dilis flour in each diet was calculated so that three levels of nitrogen intake

daily could be supplied to the rats: namely, low, 0.12 gm nitrogen/day; mid, 0.24 gm nitrogen/day; high, 0.36 gm nitrogen/day.

Nitrogen determination made on the raw and deodorized dilis flour and casein gave the following values:

	Nitrogen Per cent	Protein (N x 6.25) Per cent
Raw dilis flour	12.66	79.12
Deodorized dilis flour	12.27	76.69
Casein	15.63	97.68

On the basis of the above nitrogen content of both types of dilis flour and casein, the nine different diets were prepared having the percentage composition listed in Table 1. The amount of fish flour was varied to supply the three nitrogen

TABLE 1.—Composition of diets.

Ingredients	Casein (control)			Raw dilis flour			Deodorized dilis flour		
	0.12 gm N/day	0.24 gm N/day	0.36 gm N/day	0.12 gm N/day	0.24 gm N/day	0.36 gm N/day	0.12 gm N/day	0.24 gm N/day	0.36 gm N/day
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Protein source.....	9.20	18.50	27.80	9.20	18.50	27.80	9.20	18.50	27.80
Cerelose.....	76.97	67.67	58.37	76.97	67.67	58.37	76.97	67.67	58.37
Salka flax (cellulose).....	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vegetable oil.....	6.70	6.70	6.70	6.70	6.70	6.70	6.70	6.70	6.70
Salt mix.....	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Choline chloride.....	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mix.....	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

levels at the expense of the carbohydrate (cerelose). All other ingredients were provided for in the same quantities in all of the diets prepared. Tables 2 and 3 give the composition of the salt mix and the vitamin mix. The salt mix was identical to that used by Lichstein, et al.(7) The vitamin mix was identical to that used by Tillman.(10) In compounding the diets, the vi-

TABLE 2.—Composition of salt mix.

Salt	Amount
	mg/100 gm
Na Cl.....	600.0
K ₂ HPO ₄ .3H ₂ O.....	1,516.0
Ca ₅ H ₂ (PO ₄) ₃ .4H ₂ O.....	340.4
MgSO ₄ .7H ₂ O.....	365.6
Fe(C ₆ H ₅ O ₇) ₂ .6H ₂ O.....	100.0
CaCO ₃	1,076.0
KI.....	28.0
MnSO ₄ .6H ₂ O.....	1.2
ZnCl ₂	0.9
CuSO ₄ .5H ₂ O.....	1.1

tamins were weighed on an analytical balance and the remaining components were weighed on an Ohaus balance. The ingredients were thoroughly mixed in a large Hobart mixer. The diets were stored in a refrigerator.

TABLE 3.—Composition of vitamin mix.

Vitamin	Amount
	mg/100 gm
Thiamine HCL, mg	0.5
Riboflavin, mg	0.8
Niacin, mg	4.0
Pyridoxine HCL, mg	0.5
Ca—Pantothenate, mg	4.0
Biotin, mg	0.04
Beltic acid, mg	0.2
Menadione, mg	0.5
Vitamin B ₁₂ , mg	0.003
Inositol, mg	10.0
p-Aminobenzoic acid, mg	10.0
Vitamin E, mg	10.0
Vitamin A, IU	2,000.0
Vitamin D, IU	200.0

Growth studies.—For growth studies, 27 rats were kept in groups of three in animal cages and fed ad libitum at 0.12 gm, 0.24 gm, and 0.36 gm nitrogen intake per day on the casein, raw and deodorized dilis flour diets.

For metabolism studies, 18 additional rats were housed individually in metabolism cages. Three rats of similar weight were fed the same diet of nitrogen as that of the rats above. However, only raw and deodorized dilis flour were fed to these rats, ad libitum.

The study covered forty-five days, divided into five successive periods of ten days each, the last period with only five days.

All animals which were observed visually daily, were weighed every five days. Urine and feces were collected daily from the animals in metabolism cages. The urine for each 24-hour period was acidified, brought to volume, and stored under toluene. Collections for each 10-day period were made into a composite from which aliquots were taken for nitrogen determinations. The feces collected for each 10-day period were made into a composite, weighed, ground in a mechanical grinder, and aliquots were taken for nitrogen determinations. Nitrogen determinations by the macro-Kjeldahl method of the AOAC(1) were made in duplicate on each 10-day urine and fecal composite for each of the 6 groups of 3 rats each. Feeds, urine, and feces were all kept refrigerated during the study.

At the end of the 45-day period of the study, all animals were examined as to their fur, teeth, eyes, and general appearance before being sacrificed. One rat was autopsied to examine visually the subcutaneous tissue, muscle, and condition of the internal organs, especially the liver and the alimentary tract.

RESULTS

Weight gains and food intakes.—Table 4 shows the food intake and efficiency of food utilization by animals fed on raw dilis flour; Table 5 shows that of animals fed on deodorized dilis flour. The data are presented separately for each five periods of the study with an over-all mean for the 45-day study.

TABLE 4.—*Weight gain, food intake and efficiency food utilization of rats on metabolism study fed with raw dilis flour for 45 days.*

Period and level of nitrogen intake	Average weight gain *	Average food intake *	Food efficiency *
<i>gm nitrogen/day</i>	<i>gm/day</i>	<i>gm/day</i>	
Period I:			
0.12	2.80	6.7	0.41
0.24	3.40	6.9	0.49
0.36	3.66	7.9	0.45
Period II:			
0.12	3.08	10.6	0.29
0.24	3.67	10.6	0.34
0.36	3.61	10.6	0.34
Period III:			
0.12	2.58	12.0	0.21
0.24	2.81	12.6	0.22
0.36	2.65	12.6	0.21
Period IV:			
0.12	1.72	11.1	0.15
0.24	1.97	12.2	0.16
0.36	1.99	12.2	0.16
Period V:			
0.12	2.26	9.6	0.23
0.24	1.58	9.7	0.16
0.36	1.64	9.6	0.17
Mean for 45 days:			
0.12	2.51	10.0	0.25
0.24	2.87	9.2	0.31
0.36	2.89	10.6	0.26

* Mean of 3 rats on each diet.

Average gain in weight seems to vary little within the three levels of nitrogen in the case of the rats fed with raw and with deodorized dilis flour. As a rule, the rats fed at the low nitrogen level gained less than those fed at the mid or the high levels of nitrogen. The highest gain in weight was 5 grams per day in period I for the rats which received deodorized dilis flour at the 0.36 gm nitrogen per day intake. The average food intake was 7.6 grams of deodorized dilis flour, which gave the highest food efficiency ratio of 0.65. The average weight gain increased

markedly up to period III and then gradually declined, together with the average food intake, in periods IV and V. From the gain in body weight per gram of food intake, the food efficiency ratios were calculated. The food efficiency ratio was generally a little higher in the rats fed at the mid and high levels of nitrogen. Food efficiencies of 0.49 in the raw dilis and 0.65 in the deodorized flours were highest in period I. Then the food efficiency gradually decreased and was lowest at period V when the active growth period had passed.

TABLE 5.—*Weight gain, food intake and efficiency of food utilization of rats on metabolism study fed with deodorized dilis flour for 45 days.*

Period and level of nitrogen intake	Average weight gain *	Average food intake *	Food efficiency *
<i>gm nitrogen/day</i>	<i>gm/day</i>	<i>gm/day</i>	
Period I:			
0.12	3.36	7.3	0.46
0.24	4.74	7.6	0.62
0.36	5.00	7.6	0.65
Period II:			
0.12	2.04	10.8	0.19
0.24	2.06	10.8	0.19
0.36	2.32	10.8	0.21
Period III:			
0.12	4.41	10.8	0.40
0.24	3.45	13.6	0.25
0.36	3.10	14.3	0.21
Period IV:			
0.12	2.00	12.5	0.16
0.24	2.02	11.9	0.17
0.36	2.27	13.7	0.17
Period V:			
0.12	0.94	10.8	0.09
0.24	0.44	10.3	0.04
0.36	1.6	11.	0.14
Mean for 45 days:			
0.12	2.73	10.5	0.26
0.24	2.77	10.8	0.26
0.36	2.99	11.4	0.26

* Mean of 3 rats on each diet.

Growth response.—Rats on the dilis diet gave a growth response varying from the lowest, 64.9 per cent, to the highest, 163.2 per cent of the weight gained by the control rats fed on the same nitrogen level with casein as the protein source. This is shown in Table 6. There was no uniformity in the noted average weight gain per day by the rats on either the casein or the dilis diets, during the five periods, but there was a definite trend in gaining more weight from the beginning to the middle periods and lesser gains towards the later periods. Per-

centage gains by rats on the dilis diets were frequently above or close to the gains made by rats on the casein diet which served as control.

Figures 1 to 3 represent the mean growth curves of the rats fed at the three levels of nitrogen from casein, raw, and deodorized dilis flour. Rats fed at the low level had the lowest growth curve, in all three diets. At the end of the study there was a difference of 26 to 39 grams in the mean weight of the rats on the low nitrogen level and those on the high level.

TABLE 6.—*Growth response of rats.*

Rats on growth studies	Grams nitrogen per day								
	Casein control			Raw dilis flour			Deodorized dilis flour		
	0.12	0.24	0.36	0.12	0.24	0.36	0.12	0.24	0.36
Period I:									
Mean wt. gain, gm/day	2.1	3.6	4.6	2.6	3.8	3.8	2.4	3.0	3.5
Wt. gained by control rats, per cent.	100.0	100.0	100.0	126.3	103.8	82.8	119.0	82.7	75.4
Period II:									
Mean wt. gain, gm/day	3.1	2.5	4.6	3.8	3.5	3.2	2.9	3.9	4.0
Wt. gained by control rats, per cent.	100.0	100.0	100.0	123.9	140.8	68.7	94.8	155.6	87.8
Period III:									
Mean wt. gain, gm/day	3.7	3.9	2.5	3.1	4.3	4.1	3.1	3.4	2.7
Wt. gained by control rats, per cent.	100.0	100.0	100.0	83.5	111.9	163.2	85.1	88.6	104.7
Period IV:									
Mean wt. gain, gm/day	2.5	2.1	1.5	2.3	1.8	2.2	1.9	2.0	1.6
Wt. gained by control rats, per cent.	100.0	100.0	100.0	91.5	88.8	147.4	76.6	99.0	107.9
Period V:									
Mean wt. gain, gm/day	1.6	1.5	1.2	2.0	1.3	0.9	1.4	1.0	1.0
Wt. gained by control rats, per cent.	100.0	100.0	100.0	68.9	83.1	76.7	87.3	64.9	83.3
Total, 45 days:									
Mean wt. gain, gm/day	2.8	2.7	3.3	2.8	2.9	2.8	2.3	2.7	2.6
Wt. gained by control rats, per cent.	100.0	100.0	100.0	99.0	106.0	108.0	85.0	101.0	98.0

Figures 4 to 6 represent the mean growth curves of rats on the dilis flour compared with the growth of rats on casein at the same level of nitrogen intake. The three curves in Figure 4 are close, with the raw and deodorized dilis flour showing a greater gain than the control especially in the first half of the study at the low level of nitrogen intake. Figures 5 and 6 show

that growth on the control and raw dilis flour diets was similar throughout the study, while growth on the deodorized flour was stepwise rather than uniform in rate.

Figures 7 to 9, like figures 4 to 6, compare the mean growth curves of rats on dilis flour in the metabolism study with that of the rats on casein at the same levels of nitrogen. The curves for the rats on the raw and deodorized flour are near to those for the control animals with a higher growth rate for the first half of the study and then a slower rate a little below the control during the latter half.

TABLE 7.—*Nitrogen intake, excretion and efficiency of rats on metabolism studies fed with raw dilis flour for 45 days.*

Period and level of nitrogen intake	Nitrogen intake *	Nitrogen excretion *	Nitrogen retention *	Nitrogen efficiency *
<i>gm nitrogen/day</i>	<i>mg/day</i>	<i>mg/day</i>	<i>mg/day</i>	<i>mg/day</i>
Period I:				
0.12	112.4	38.9	73.6	24.9
0.24	205.3	100.5	104.8	16.6
0.36	342.1	228.8	113.2	10.7
Period II:				
0.12	177.0	42.6	134.4	17.4
0.24	316.9	198.6	118.3	11.6
0.36	459.0	372.7	86.2	7.9
Period III:				
0.12	205.8	63.1	142.7	12.5
0.24	376.7	100.9	275.8	7.5
0.36	544.8	272.6	272.2	4.9
Period IV:				
0.12	185.1	56.5	128.5	9.3
0.24	363.6	116.4	247.2	5.4
0.36	528.6	166.0	362.6	3.8
Period V:				
0.12	162.0	48.7	113.2	13.9
0.24	290.4	145.3	145.1	5.4
0.36	413.9	253.4	160.5	4.0
Mean for 45 days:				
0.12	168.5	50.0	118.5	15.6
0.24	310.6	132.3	178.3	9.3
0.36	457.7	258.7	199.0	6.3

* Mean of 3 rats on each diet.

Nitrogen intake, excretion, retention, and nitrogen efficiency.—Tables 7 and 8 present the mean nitrogen intake, excretion, retention, and nitrogen efficiency of three animals fed on each of the low, mid and high nitrogen levels of raw dilis flour and deodorized dilis flour respectively. In general, the nitrogen excretion pattern followed the nitrogen intake, being higher at higher levels of intake. The nitrogen efficiency was obtained by dividing the average weight gain per day by the corresponding intake

of nitrogen per day. The results show a higher nitrogen efficiency for both the raw and deodorized dilis flour at the low level of nitrogen intake than at the mid and high levels.

DISCUSSION

From the results obtained, the rats on both kinds of dilis flour and casein diets showed a slower growth at the low level of 0.12 gm nitrogen intake per day. This could be attributed to the insufficient amount of nitrogen they received for optional growth. The response to the mid and high levels of dilis flour compared favorably with the response to the same levels of casein control diet and a slightly better gain in weight on the dilis flour was noted in the young rats. In general the rats on the mid and high levels of nitrogen had a better food efficiency than that of

TABLE 8.—Nitrogen intake, excretion, retention and efficiency of rats on metabolism studies fed with deodorized dilis flour for 45 days.

Period and level of nitrogen intake	Nitrogen intake *	Nitrogen excretion *	Nitrogen retention *	Nitrogen efficiency *
<i>gm nitrogen/day</i>	<i>gm/day</i>	<i>gm/day</i>	<i>gm/day</i>	<i>gm/day</i>
Period I:				
0.12.....	105.1	54.7	50.3	32.0
0.24.....	207.4	92.5	114.9	22.8
0.36.....	330.0	121.7	208.3	15.1
Period II:				
0.12.....	154.9	55.4	99.5	13.2
0.24.....	294.7	153.9	140.7	7.0
0.36.....	468.0	431.8	36.2	4.9
Period III:				
0.12.....	206.2	87.9	118.2	21.4
0.24.....	370.3	225.7	114.6	9.3
0.36.....	616.7	335.3	281.4	5.0
Period IV:				
0.12.....	178.8	94.3	84.5	11.2
0.24.....	322.9	170.2	152.7	6.3
0.36.....	593.1	209.9	383.2	3.8
Period V:				
0.12.....	154.7	78.0	76.7	6.1
0.24.....	279.1	201.1	69.0	1.6
0.36.....	474.5	278.9	195.6	3.4
Mean for 45 days:				
0.12.....	160.0	74.1	85.8	16.8
0.24.....	295.0	170.5	113.5	9.4
0.36.....	496.5	275.5	221.0	6.4

* Mean of 3 rats on each diet.

the rats on the low level and this may account for their greater gain in weight. However, the nitrogen efficiency of the rats on the low level both in the raw and deodorized dilis flour diets was consistently higher than at the mid and high levels of

intake. It is apparent that the rat utilized more economically the nitrogen in the diet when the supply was limited than when it was in an abundance.

All the rats responded well to their diets. During the first two days, the rats did not eat much of the diets containing dilis flour, but from the third day on they ate voraciously from 6 to 7 grams a day at the beginning to 12 to 14 grams a day at the middle of the study. As the rat grew larger, their appetites declined a little. The rats, which were full of action, had all well formed, sharp and strong teeth, which they often sharpened by biting their wire cages. Their eyes were all brightly pink and showed no signs of anemia. Their feet and tails looked normal and showed no difference from rats on the casein control diet. The rats on the low level of nitrogen intake, however, had a somewhat rougher fur coat and a tendency to lose hair. This was not observed in those fed at the mid and high levels of nitrogen of the casein and dilis flour diets.

A rat, from the low nitrogen level dilis flour diet, which was among those sacrificed, was autopsied, and its viscera were examined. The stomach, liver and intestines were found to be normal. There was a little subcutaneous fat, and the muscles were firm.

The nitrogen metabolism and general physical appearance of the forty-five rats used in the study show that dilis fish flour has a high biological value, which is comparable to that of casein.

There was no significant difference in the results obtained with the raw and the deodorized fish flours.

The protein content of 76 to 80 per cent of Philippine-made dilis flour compares favorably with that made by Dr. Ershoff on Vio-Bin fish flour (made from various species of fish) which gives a protein content of 75 to 80 per cent, as reported by Levin.⁽⁶⁾

SUMMARY

The biological quality and food efficiency of Philippine-made fish flour was studied with the use of forty-five female weanling rats. Two forms of dilis flour, raw and the deodorized, were used with casein serving as control. Diets adequate with regards to vitamins, minerals, fat and carbohydrate content were used.

The only item of the diet present in varied amounts was the protein source, which was supplied at three levels, the low at 0.12 gm nitrogen per day, the mid at 0.24 gm nitrogen per day, and the high at 0.36 gm nitrogen per day. The study was carried on for 45 days and was divided into five periods. Growth response, food efficiency and nitrogen metabolism results were recorded.

The results obtained show that dilis fish flour both in raw and deodorized form is biologically adequate and can support normal growth in rats. As a dietary source of good quality protein, dilis flour is comparable to casein, producing even a better growth response in the young animal at a low level of nitrogen intake. For a larger growth response, however, the fish flour, when used as the sole protein source, should be provided at 15 to 25 per cent of the protein in the diet.

ACKNOWLEDGMENT

This work was done at the Oklahoma State University at Stillwater where the senior author held an Ida H. Hyde International fellowship in 1956-1957 granted her through the American Association of University Women. Her special thanks go to Mrs. Florence S. Waddill, Mr. George Odell, Mrs. Ruby Moore, Miss Gwen Slover, Dr. Dorothy Steel, Miss Esther Winterfeldt, and Mrs. Valencia Stone, all of this University, for many forms of assistance they rendered her.

The fish flours used in the study were generously provided for by the Philippine Institute of Fisheries Technology, Manila.

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ILLUSTRATIONS

PLATE 1

FIG. 1. Mean growth of rats on diets with three different levels of casein

2. Mean growth of rats on diets with three different levels of raw dilis flour.
3. Mean growth of rats with different levels of deodorized dilis flour.
4. Mean growth curves of rats on growth study fed 0.12 gm nitrogen level as compared to casein.
5. Mean growth curves of rats on growth study fed 0.24 gm nitrogen level as compared to casein.
6. Mean growth curves of rats on growth study fed 0.36 gm nitrogen level as compared to casein.
7. Mean growth curves of rats on metabolism study fed 0.12 gm nitrogen level as compared to casein.
8. Mean growth curves of rats on metabolism study fed 0.24 gm nitrogen level as compared to casein.
9. Mean growth curves of rats on metabolism study fed 0.36 gm nitrogen level as compared to casein.

ON THE ARTIFICIAL HATCHING OF TRICHURIS VULPIS EMBRYONATED EGGS WITH A NOTE ON ABNORMAL OVA AND POSTURE OF THE EMBRYOS

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TWO PLATES

Since the publication of my initial paper(3) on the extra-corporeal hatching of embryonated ova of *Trichuris vulpis*, additional data have accumulated which form the subject of the present article. Some of the observations herein recorded have already been reported in abstract in one of the meetings of the American Society of Parasitologists.

MATERIALS AND METHODS

The materials and methods employed consisted briefly in the collection of the eggs by sedimentation and floatation, then embryonation, and finally hatching in suitable Petri dishes. All materials were sterilized to control fungus and ciliate contamination. In one series formalin in the concentration of 0.05 per cent was used in addition. Sterile distilled water instead of tap water was also used for sedimentation and embryonation. Cultures were observed longer than previously. The procedure is described in detail in my first article.

RESULTS AND DISCUSSION

The results are indicated in Tables 1 and 2. Table 1 shows a wide variation in the hatching of the embryonated ova of *Trichuris vulpis*, the highest being 81.98 per cent noted in trial 8 on the tenth day, the lowest being 0.51 per cent in trial 5 on the seventh day. It is also evident that there were days when hatching seemed to have been delayed or suspended entirely as noted in trials 1 through 6 and 7. Trials 8, 9 and 10 gave the best hatching percentage. Further, there seemed to be a daily fluctuation in the hatching of the ova. In general, cultures contaminated with fungus growths and, to lesser extent, ciliates tended to favor higher hatching percentage. This circumstance lends support in part to previous observation. The present

hatching record is approximately three times higher than the highest ever attained artificially.(3) It appears that ova embryonated in sterile distilled water and later transferred to bile-Locke's solution for hatching had decidedly increased the hatching percentage of the eggs of *Trichuris vulpis*. In general, the record presented in Table 2 shows that embryonated ova will hatch to a considerable extent irrespective of the fungus and ciliate contamination. Higher percentage of hatch was noted

TABLE 1.—Hatching record of *T. vulpis* embryonated ova in culture of bile-Locke's solution.

Trial number	Days after preparation of culture							
	1	2	3	4	5	6	7	8
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1.....	0	0	5.50	3.38	-----	8.10	4.95	5.00
2.....	0.78	0	1.70	1.60	-----	1.78	0.94	1.20
3.....	0	2.85	2.35	2.48	5.55	2.94	2.85	5.40
4.....	0	0	36.76	39.51	41.64	36.73	32.52	36.33
5.....	0	0	0.80	0.74	0	1.55	0.51	-----
6.....	0	0.83	0.91	2.60	0	1.92	2.56	3.76
7.....	0	0	9.77	6.20	6.19	9.32	8.25	4.50
8.....	27.25	40.75	46.09	54.87	49.79	73.88	65.95	71.57
9.....	18.53	17.22	47.21	35.25	37.54	58.11	60.94	65.97
10.....	15.32	41.52	55.70	55.02	70.85	56.61	60.65	59.46
Average.....	6.19	10.32	20.68	22.22	26.44	25.09	24.01	28.13

Trial number	Days after preparation of culture			Remarks
	9	10	11	
	Per cent	Per cent	Per cent	
1.....	6.66	-----	-----	Few cysts of ciliates.
2.....	0.64	-----	-----	-----
3.....	6.21	9.79	5.44	Moderate fungus growth; few cysts of ciliates.
4.....	34.78	40.87	30.12	-----do-----
5.....	-----	1.87	-----	-----
6.....	3.52	3.92	1.77	Abundant fungi.
7.....	7.14	16.60	10.00	-----do-----
8.....	76.59	81.98	-----	-----do-----
9.....	68.39	62.46	-----	Abundant fungus growth; many ciliates.
10.....	62.85	61.36	-----	Abundant fungi.
Average.....	29.64	34.85	11.83	-----

in plain distilled water than those to which formalin was added, the highest in the former being 49.33 per cent observed in trial 3 on the seventh day, while in the latter 4.15 per cent in trial 4 on the fifth day. From the above findings, one can assume that formalin has a decided deterrent effect in the hatching of the embryonated ova. In the first as in the second series, there was a daily fluctuation in the hatching percentage of the ova. Although the highest percentage of hatch was noted in the bile-

Locke's solution, it was by no means essential to artificial hatching, since in plain sterile distilled water the embryonated ova showed also a reasonable high degree of hatch to the extent of 49.33 per cent, surpassing the data of 28 per cent previously recorded.

During the investigation, unusually large and perfectly formed eggs were noted. While some failed to develop, embryonation took place in others. The average measurement of an oversized egg was 0.106 × 0.05 mm; normal egg 0.075 × 0.04 mm (Plate 1, figs. 1 and 2). Rubin(2) encountered an exceptionally large egg measuring 0.105 × 0.04 mm which, however, failed to em-

TABLE 2.—Hatching record of *T. vulpis* embryonated ova in cultures of distilled water and distilled water with formalin.

Trial number	Distilled water					Distilled water with formalin				
	Days after preparation of cultures									
	1	3	5	7	9	1	3	5	7	9
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1-----	17.70	19.93	10.71	13.27	20.28	0	0.47	0.47	0.50	0.70
2-----	6.92	14.75	13.54	6.93	8.80	0.95	0	2.15	0.50	0.33
3-----	28.37	46.66	39.21	49.33	38.13	0.85	4.00	0.47	0.27	0.25
4-----	6.17	13.39	11.81	16.66	25.78	2.30	3.95	4.15	0.20	1.75
5-----	0.94	1.00	0.80	1.20	2.00	4.15	2.60	2.56	0.39	0.40
6-----	3.00	2.00	2.50	2.00	1.00	0	0.80	0.88	1.33	0.80
7-----	2.00		2.43	2.50	3.56	0	0	0.87	0	0.24
8-----	0.55	0.70	1.20	2.20	1.25	0	0.86	0.56	0.58	0.24
9-----	7.20	8.00	5.55	6.15	5.00	0.86	1.25	0.85	0	0.22
10-----	5.58	4.46	3.85	5.25	6.00	0	0	0.65	0	0.20
Average-----	7.84	12.32	9.16	10.55	11.18	0.91	1.39	1.36	0.38	0.51

bryonate. On the other hand, one undersized and perfectly-formed egg was also noted with a measurement of 0.055 × 0.025 mm. The deformed eggs assumed varied shapes and sizes, ranging from spherical to roughly triangular, spindle or lobulated (Plate 1, figs, 3 to 7, 11, and 12). The irregularly shaped ova were more than the number presented in Plate 1. The pluggs of the abnormally shaped eggs were intact, but were reduced in number in some or even totally lost in others. Twin-shelled eggs were also encountered, either side to side or end to end fusion (Plate 1, figs. 8, 9, and 10). As seen in Plate 1, majority of the deformed eggs were embryonated. As far as can be ascertained, the occurrence of so many irregular shapes and sizes of the ova of *Trichuris vulpis* has never attracted attention; yet this is quite remarkable and even outranks the number of malformed and unfertilized ova of *Ascaris lumbricoides* recorded by Otto.(1)

Two ways of exit of the embryo from the shell have been described; namely, through one of the poles of the egg with the anterior end emerging first, and through a rent in the shell wall with the pluglike knobs in situ (Yutuc). In this study, a number of unusual hatching postures of the embryos were encountered. Some of the embryos observed showed the middle segment doubled up, escaping first out of one pole of the shell (Plate 2, figs. 1 and 2). The embryo in its attempt to free itself was able to push through the other opening with the anterior end, while the middle segment and the posterior end remained inside the shell (Plate 2, fig. 3). Further efforts invariably ended to a "ringed" embryo (Plate 2, fig. 4). In some, the embryo was able to extricate itself partly by disengaging the tail end first, the anterior end remaining enclosed by the shell (Plate 2, fig. 5). Another unusual hatching consisted in the embryo emerging simultaneously the anterior end in one pole, while the posterior in the opposite opening, resulting in a "ringed" embryo (Plate 2, figs. 4 and 6). The last form, which was quite rare, consisted in the anterior and posterior ends of the embryo emerging at the same time through one of the apertures of the shell (Plate 2, fig. 7). Essentially all these variations in the hatching postures belong to the first form of exit already described, but the postures of the embryo are considered unusual.

SUMMARY

Embryonation of whipworm eggs in sterile distilled water enhanced considerably the percentage of hatching in the bile-Locke's solution contaminated mostly with fungus growth. Embryonated ova hatched also in pure distilled water and in distilled water with formalin free of fungus and ciliate growths. Higher percentage of hatch was noted in the former than in the latter, the highest, however, being in the bile-Locke's solution.

Over- and under-sized as well as mal-formed eggs and unusual hatching posture of the embryos were noted and described.

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ILLUSTRATIONS

PLATE 1

FIG. 1. *Trichuris vulpis* normal embryonated egg.

2. Oversized egg.

3-7. Malformed embryonated eggs.

8-10. Twin-shelled eggs with one partly embryonated.

11 & 12. Oversized and malformed eggs which failed to embryonate.

PLATE 2

FIG. 1.-2. Embryos escaping out of the shell with middle segment doubled up.

3. Embryo with the anterior end free but tail end and part of the middle segment still in the shell.

4. "Ringed" embryo.

5. Embryo with the posterior out of the shell first with anterior end enclosed.

6. Embryo emerging with both the anterior and posterior ends through opposite opening of the shell.

7. Embryo with the anterior and posterior ends escaping simultaneously through one of the poles of the shell.

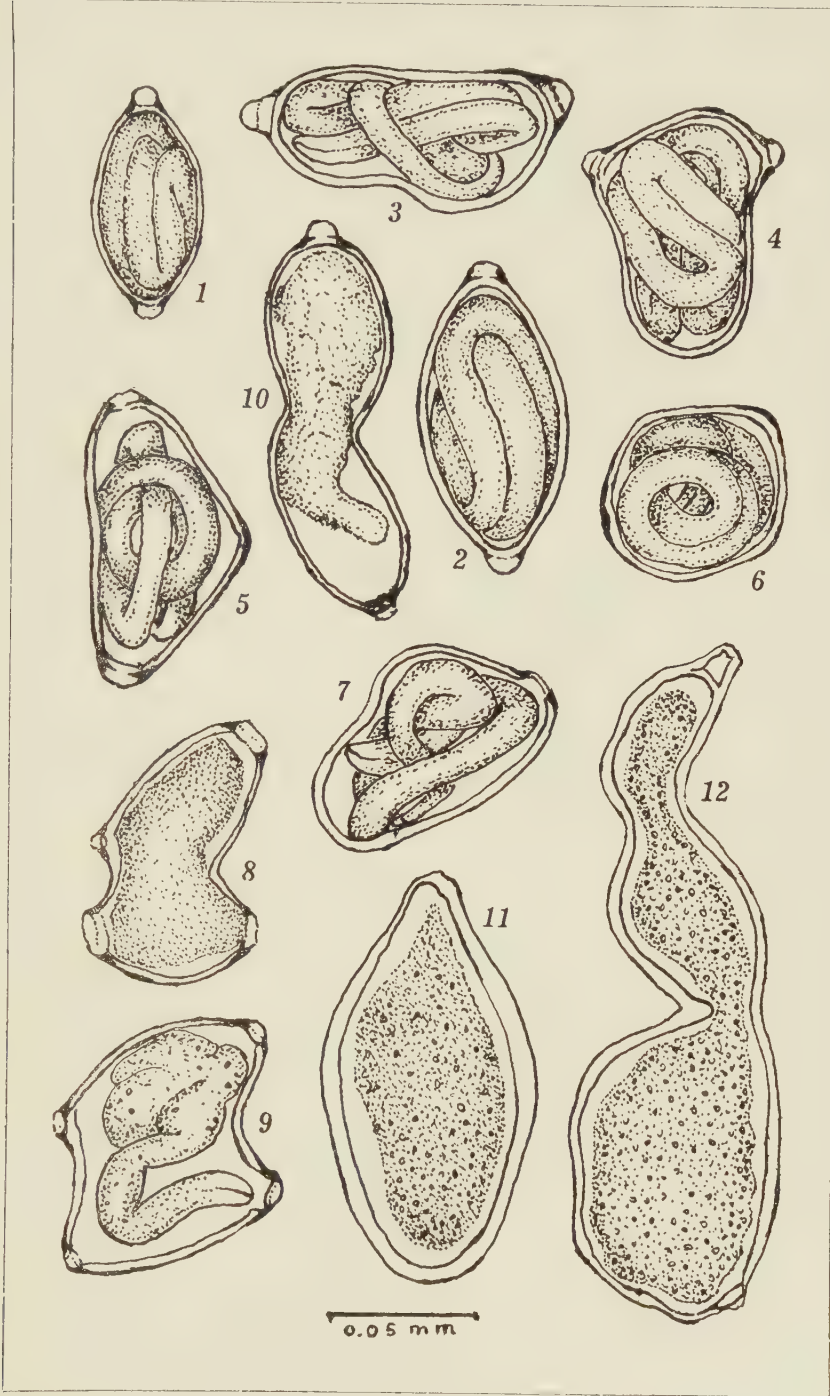


PLATE 1.

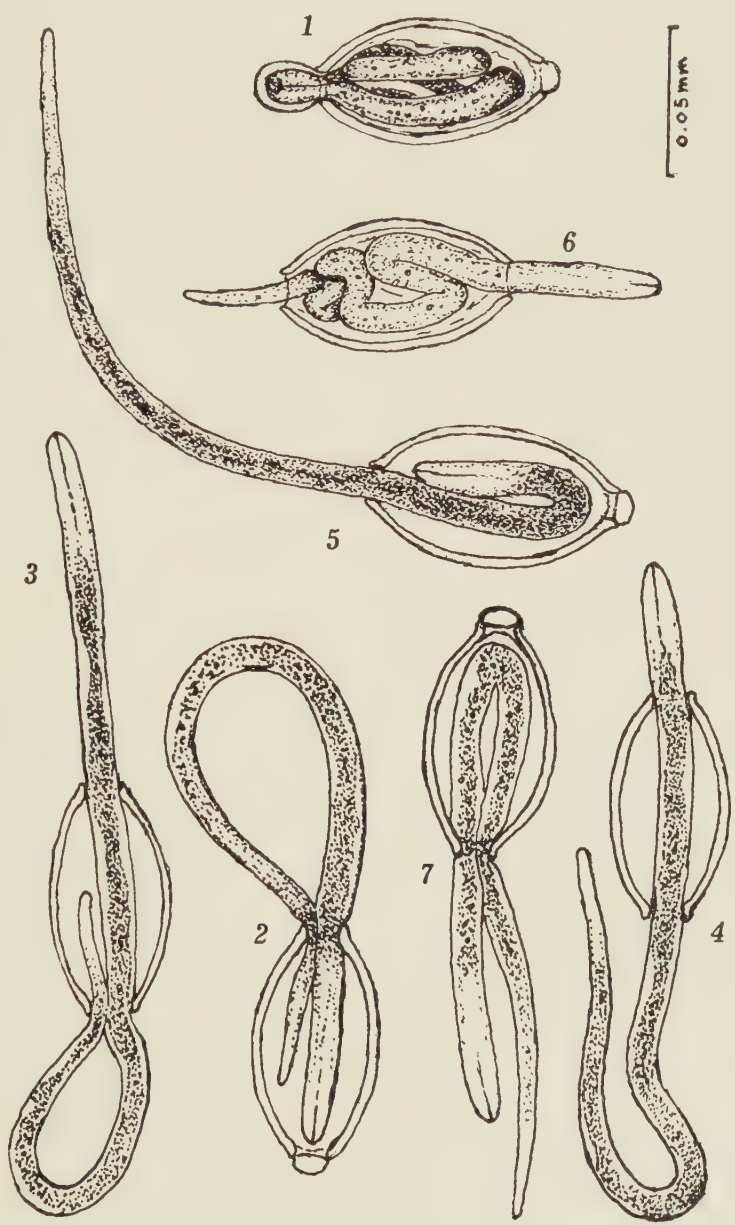


PLATE 2.

RECORDS AND DESCRIPTIONS OF JAPANESE TIPULIDÆ (DIPTERA), PART VI

THE CRANE-FLIES OF HONSHU, II

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EIGHT PLATES

The present report on the crane-flies of Honshu brings the cumulative list for the island to 96 species, with a total of 350 species treated in the six parts so far prepared under this general title. As before, particular stress is placed on the describing and illustrating of species that have not been discussed or shown hitherto. At this time, species of the two tribes Pediciini and Eriopterini are considered. As was the case in the preceding report, the materials here discussed were taken in greatest part by Dr. Kintaro Baba, of Kurokawa, to whom my continued thanks and appreciation are extended.

RECORDS OF DISTRIBUTION

PEDICIINI

PEDICIA Latreille

Pedicia LATREILLE, Gen. Crust. et Ins. 4 (1809) 255.

As has been stressed by the present writer in various other papers, the species of the typical subgenus *Pedicia* as known from the Japanese Empire present unusual difficulties, particularly in those species that tend to bridge the gap between the typical group and *Tricyphona*. The group of forms that center around *Pedicia* (*Pedicia*) *grandior* have venational characters that approach closely those of various unquestioned members of the subgenus *Tricyphona*. I am providing a short preliminary discussion concerning these two major groups of the genus.

Subgenus *Pedicia* Latreille. The regional members of the subgenus may be separated roughly into two groups, based on venational characters.

1. Wings with r-m at or beyond the fork of Rs, in the latter case R_{4+5} short to very short, with two sections; cord oblique; cells 1st M_2 short-pentagonal. Included species *P. (P.) brachycera* Alexander (Honshu), *P. (P.) cockerelli* Alexander (Eastern Siberia, Karafuto), *P. (P.) daimio* Matsumura (Hokkaido),

P. (P.) gifuensis Kariya (Honshu), *P. (P.) lætabilis* Alexander (Korea), *P. (P.) nawai* Kariya (Honshu), and *P. (P.) subfalcata* Alexander (Eastern China).

2. Wings with r-m before the fork of Rs, R_{4+5} thus entire, longer than r-m; cord more nearly transverse; cell 1st M_2 more elongate, pentagonal or hexagonal in outline. Included species *P. (P.) cubitalis* Alexander (Honshu), *P. (P.) gaudens* Alexander (Honshu), *P. (P.) grandior* Alexander (Honshu), and *P. (P.) issikiella* Alexander (Shikoku).

3. Intermediate in characters between the above groups; r-m beyond the fork of Rs on R_{4+5} , as in Group 1, but cell 1st M_2 elongate and cord subtransverse. Included species *P. (P.) subtransversa* Alexander (Honshu).

Subgenus *Tricyphona* Zetterstedt. In his last treatment of the British Tipulidæ, Edwards (1938)¹ recognized the genus *Pedicia* Latreille, with four subgenera, including besides typical *Pedicia* (type, *rivosa* Linnæus), *Crunobia* Kolenati (type, *schineri* Kolenati), *Amalopsis* Haliday (type *occulta* Meigen), and *Tricyphona* Zetterstedt (type, *immaculata* Meigen). I still do not believe it to be advisable to attempt to split the basic subgenus *Tricyphona* into lesser groups since it is certain that when the World fauna is considered so many additional groups of comparable value are to be found that it would seem illogical not to provide names for them and it would be found to be almost impossible to distinguish between such groups except on very trivial and merging characters.

In the Japanese fauna, several species of the *occulta* group (*Amalopsis* Haliday) are found, some of which are discussed later. In addition to the venational character mentioned by Edwards of a very deep and sessile cell M_3 , with m-cu connecting with M_4 , it may be indicated that vein R_{2+3+4} is present and is unusually erect; cell M_2 is open or closed in the different species. It may be noted further that some Nearctic species that center about *vernalis* Osten Sacken have the medial and cubital fields very much as in *occulta* and allies but the radial field is quite distinct in the long vein R_{4+5} . The nature of the shifting of the veins at the end of Rs and the transfer of vein R_4 from the generalized position on the posterior fork of the sector onto the upper fork to produce an element R_{2+3+4} is one of the most striking features in the venation of the *Pediciini*.

¹ Edwards, F. W. British short-palped crane-flies. Taxonomy of adults. Trans. Soc. British Ent. 5 (1938) 1-168, 31 figs., 5 pls.

Any student working on the group should become familiar with the problem which has been discussed in the following references.²

29 (291). *PEDICIA (PEDICIA) BRACHYCERA* Alexander, ... Plate 1, fig. 1.

Pedicia brachycera ALEXANDER, Philip. Jour. Sci. 50 (1933) 145-146, pl. 1, fig. 12 (venation), pl. 3, fig. 40 (♀ antenna).

Pedicia brachycera KARIYA, Philip. Jour. Sci. 53 (1934) 304.

The unique type was from Shirouma-dake, Shinano, taken August 8, 1931, by Machida.

ECHIGO: Kajiya, Nechi, Itoyogawa, August 18, 1954 (*Yoshitomo Maruyama*); Baba No. 36.

Wings with the darkened seam along distal section of vein Cu_1 not reaching the margin. Male hypopygium (Plate 1, fig. 1) with the posterior border of the tergite only moderately produced, subtruncate, with sparse small setæ, the margin fringed with delicate setulæ. Basistyle, *b*, stout, the interbase, *i*, an unusually short, suboval darkened blade. Dististyle, *d*, large, roughly suboval in outline, the outer margin with about eight small spines; beak very obtuse, with a small sclerotized flange near its base; mesal face of style with a low lobe that is densely set with slender spinous points, those nearer the base becoming very small; disk of style with numerous small spinous setæ, with a few very long bristles near outer end. Phallosome small, the gonapophyses shorter than the ædeagus, the obtusely rounded tips microscopically crenulate.

30 (292). *PEDICIA (PEDICIA) CUBITALIS* Alexander. Plate 1, fig. 2.

Pedicia (Tricyphona) cubitalis ALEXANDER, Philip. Jour. Sci. 51 (1933) 396-397, pl. 1, fig. 16 (venation).

The type, a unique female, was from Mount Kurobegoro, Etchu, taken in a subalpine meadow, altitude 7,800 feet, August 10, 1931, by Imanishi. Two additional males are here recorded, one of which is designated as allotype of the species.

Male.—Length, about 23 to 24 mm; wing, 21 to 23 mm; antenna, about 1.6 to 1.9 mm.

² Alexander, C. P. A new interpretation of the wing-venation of the Pediciine crane-flies (Tipulidæ, Diptera). Ent. News 29 (1918) 201-205, 1 pl.

Alexander, C. P. The interpretation of the radial field of the wing in the Nematecerous Diptera, with special reference to the Tipulidæ. Proc. Linn. Soc. New South Wales 52 (1927) 42-72, 22 figs.

Alexander, C. P. A comparison of the systems of nomenclature that have been applied to the radial field of the wing in the Diptera. 4th Internat. Congress Ent. 2 (1929) 700-707, 3 pls.

Antennæ 13-segmented; flagellar segments crowded, shorter than the verticils, outer three or four segments much smaller. Head with vertical depression relatively shallow, subcircular in outline. Mesonotal præscutum with four darker stripes, the intermediate pair narrow, very vaguely bordered by darker; vestiture of interspaces white, conspicuous. Legs longer than in female. Wings with the dark pattern more as in *gaudens*, excepting the narrow brown seam along vein Cu; central area of disk paler than the broad border; cell R_1 extensively darkened.

Abdominal tergites fulvous, with a conspicuous brown central stripe, wider and slightly darker behind; lateral borders narrowly pruinose; basal sternites light yellow, the outer segments, including the hypopygium, passing into brownish black. Male hypopygium (Plate 1, fig. 2) with the median area of the tergite somewhat produced, the outer border nearly truncate to very gently emarginate. Basistyle, *b*, with the interbasal blade weakly dilated on outer half. Dististyle, *d*, with an unusually heavy armature of spines, including a row of about 15 major spines along the outer margin, becoming more numerous but not larger at outer end of row; a second series of smaller spines down face of beak near inner margin, totaling about 12 to 15, the more basal ones more slender; lower beak with the usual elongate welt or modified area, provided with numerous strong setæ. Ædeagus terminating in a decurved black head.

Allotype, male, Mount Ontake, Hida, July 6, 1934 (*H. Ise*); through the kindness of Kariya.

31 (293). *PEDICIA* (*PEDICIA*) *GAUDENS* (Alexander).

Plate 1, fig. 3.

Tricyphona gaudens ALEXANDER, Ent. Soc. America Ann. 17 (1924) 440-441.

The type was from the vicinity of Kyoto, taken in May, 1923 by Takeuchi. Other records include Mount Daisen, Hoki, July 1, 1931 (Tokunaga); Takane, Kuga-gun, Nagato, May 6, 1930 (Teranishi).

This interesting fly was described as a *Tricyphona* and most nearly approximates this subgenus of any known member of the present subgenus. The darkened seam along vein Cu of the wings is entirely lacking. Venation: r-m moderately oblique, connecting with R_s at or shortly before fork; cell 1st M_2 elongate, pentagonal in outline, subequal in length to the distal section of vein M_3 ; petiole of cell M_1 variable in

length, in the type shorter than m, in other specimens much longer, up to twice the length of m; m-cu at or shortly beyond the fork of M.

Antennæ 13-segmented; basal two segments black, flagellum fulvous. Male hypopygium (Plate 1, fig. 3) with the posterior border of tergite strongly produced into a subquadrate lobe, its posterior margin truncate or virtually so. Basistyle, *b*, with the outer lobe stout, conspicuously setiferous, some of the bristles very long; interbase expanded into a broad blade. Dististyle, *d*, strongly fused with the apex of the basistyle, the suture indicated on the lateral part; style yellow, the beak short and stout; outer margin with about eight small spines, those nearest the beak a little larger; near margin of style on side of beak with a further concentration of eight much smaller spines; elongate welt of the lower beak with numerous strong spinous setæ.

32 (294). *PEDICIA (PEDICIA) GIFUENSIS* Kariya.

Pedicia gifuensis KARIYA, Philip. Jour. Sci. 53 (1934) 305-306, pl. 1, fig. 2 (wing), 6 (♂ hypopygium), 7 (antenna), 8 (ovipositor).
Pedicia gifuensis ESAKI, et al., Icon. Insect. Japon. Ed. 2 (1950) 1520 fig.

The type was from Mount Kinkwa, Hida, taken from April 13 to May 27 and again in August and September.

ECHIGO: Kurokawa, June 12, 1953 (*Kintaro Baba*).

A large and showy species with the darkened wing pattern heavy and conspicuous, the dark seam along vein Cu broad, at margin expanded so as to include also the end of vein 1st A. Fore femora extensively blackened, with only the bases yellow. I do not possess a male which, from the original description and figure, is distinctive in the nature of the dististyle; this bears a sole-shaped welt on the lower face, the armature of the polished apex consisting only of three or four minute spines.

The subspecies *Pedicia (Pedicia) gifuensis sawadai* Kariya, taken at Hakone, where it flies in May, is generally similar to the typical form, having the dark seam along Cu not expanded at tip and the darkened area at origin of Rs not quite reaching vein M.

33 (295). *PEDICIA (PEDICIA) GRANDIOR* (Alexander).

Tricyphona grandior ALEXANDER, Ent. Soc. America Ann. 16 (1923) 69-70.

Tricyphona grandior ESAKI, et al., Icon. Insect. Japon. Ed. 2 (1950) 1520, fig.

Pedicia (Tricyphona) grandior ALEXANDER, Philip. Jour. Sci. 82 (1953) 59, pl. 2, fig. 15 (♂ hypopygium).

The type was from Mount Hakuba, Shinano, taken July 20, 1918, by Issiki. The fly evidently is quite distinct from its nearest described relatives, *Pedicia* (*Pedicia*) *cubitalis* (Alexander) and *P. (P.) gaudens* (Alexander). This is a relatively large species (body and wing 18 mm), dull gray, the præscutum with three clearer gray stripes. Antennæ very reduced, 11-segmented. I had briefly described the dististyle in the original account and in the later reference had provided a somewhat diagrammatic figure, made from the dry type which is preserved in Taihoku, Formosa.

34 (296). *PEDICIA* (*PEDICIA*) *NAWAI* Kariya.

Pedicia nawai KARIYA, Philip. Jour. Sci. 53 (1934) 304-305, pl. 1, fig. 1 (wing), 5 (♂ hypopygium).

The type was from Mount Ontake, Hida, collected April 16, 1897, by Y. Nawa, for whom it was named. No other specimen is known to me. From Kariya's description it evidently is distinct in the small size (body and wing 17 mm), pale wing pattern, and in the structure of the male hypopygium.

The wing pattern has the costal border much paler than the other darkened areas, the humeral region yellowed, dark spot at origin of Rs circular in outline, not reaching vein M behind; darkened cubital seam reaching the margin. The venation shows r-m oblique, at fork of Rs, with R_{4+5} much shorter than r-m; cell 1st M_2 relatively short, pentagonal; m-cu at fork of M; petiole of cell M_1 longer than m. Male hypopygium with the dististyle relatively large, subrectangular in outline, with a small bladelike protuberance on inner face. Kariya does not mention the presence of marginal spines as are common in other species of the subgenus and it is presumed that these are lacking.

35 (297). *PEDICIA* (*PEDICIA*) *SUBTRANSVERSA* *SUBTRANSVERSA* Alexander.

Pedicia subtransversa ALEXANDER, Philip. Jour. Sci. 50 (1933) 146-147, pl. 1, fig. 13 (venation), pl. 3, fig. 41 (♂ hypopygium).

Pedicia subtransversa KARIYA, Philip. Jour. Sci. 53 (1934) 304.

The type male was from Shirouma-dake, Shinano, taken August 8, 1931, by Machida. Additional material has been taken in North Korea, Chonsani, Paiktusan and in the Seren Mountains, from late July to early October, 1937, by Alexander Yankovsky.

35a (297a). *PEDICIA (PEDICIA) SUBTRANSVERSA TRIACANTHA* subsp. nov. Plate 1, fig. 4.

Male.—Length, about 21 to 23 millimeters; wing, 19 to 22.

Generally as in the typical subspecies, differing as follows:

Coloration of head and thorax of holotype clear light gray, the præscutal stripes slightly darker gray, very poorly defined against the ground. In the allotype female, the ground color of the præscutum is obscure yellow, the three gray stripes distinctly separated. Knobs of halteres strongly infuscated. Tips of femora and tibiæ broadly blackened. Wing pattern much darker and more conspicuous, including the costal border which is palest immediately beyond h; in cases with a dark spot beyond the cord, in the holotype on m, in other specimens at the fork of M_{3+4} . Venation: Sc_2 variable in position, from opposite the origin of Rs to some distance before this point; r-m connecting with R_5 beyond the fork of Rs. Abdominal tergites medially chiefly fulvous or obscure yellow, the posterior borders more or less darkened, the lateral margins infuscated and more or less pruinose. Cerci strongly darkened. Male hypopygium (Plate 1, fig. 4) with only three spines on outer margin of dististyle, *d*, the outermost largest. Welt of lower beak of style with long delicate setæ on the lower or cephalic half.

Habitat.—Japan (Honshu).

Holotype, male, Mount Amakazari, Echigo, altitude 900 meters, June 26, 1955 (*Baba*); Baba No. AM 17. Allotype, female, Yoshigahira, Mount Sumon, Echigo, June 25, 1954 (*Baba*); Baba No. 35. Paratopotype, male, with the allotype.

When more abundant materials of the present fly and of typical *subtransversa* become available, this may well be found to represent a valid species.

36 (298). *PEDICIA (TRICYPHONA) CONFLUENS SUBCONFLUENS* subsp. nov. Plate 1, fig. 5, Plate 2, fig. 7.

Male.—Length about 14 to 16 millimeters; wing, 13.5 to 16; antenna, about 1.3 to 1.5.

Rostrum dark gray; palpi black. Antennæ yellowish brown, scape darker; basal flagellar segments short and crowded in the type, longer in the Nikko paratype. Head dark gray, clearer gray on front.

Pronotum dark brown, gray pruinose. Thoracic notum gray, the præscutum with three darker gray stripes; posterior sclerites and the pleura dark gray; dorsopleural membrane pale to weakly infuscated. Halteres elongate, yellow, knobs weakly

darkened. Legs with the coxæ obscure yellow; trochanters clear yellow; femora black, only the narrow bases yellow, the amount subequal on all legs; tibiæ light brown, tips darker; tarsi dark brown. Wings (Plate 1, fig. 5) brownish yellow, cell C weakly more darkened; pale brown clouds at Sc₂, origin of Rs, both ends of the pale yellow stigma, and along cord; veins brown, more brownish yellow in the prearcular and costal fields. Venation: Sc relatively short, angulated and more or less spurred near base; R₄₊₅ short to very short, cell R₄ thus very short-petiolate to subsessile; cell M₂ open, as in the typical form; m-cu at or just beyond the fork of M.

Basal abdominal tergites obscure yellow, with a conspicuous black central line, the sixth and succeeding segments uniformly brownish black; basal sternites light yellow. Male hypopygium (Plate 2, fig. 7) with the tergite transverse, the posterior border produced into two low lobes, separated by a shallow U-shaped emargination, the lobes densely setuliferous, the margin and dorsal surface of plate with long coarse setæ. Basistyle, *b*, relatively short and stout, the low outer lobe and dorsal face with long coarse black setæ; interbase, *i*, a long slender horn or rod that narrows very gradually to the acute tip, the surface glabrous. What appears to represent a single large dististyle, *d*, occupies the outer end of style, including an outer narrow blade or spine and two much broader flattened blades, the larger intermediate one with long erect pale setæ along its inner face and margin; inner blade narrower, its lower surface with abundant shorter and stouter setæ.

Habitat.—Japan (Honsu).

Holotype, male, Mount Amakazari, Echigo, altitude 1,000 meters, June 25, 1955 (*Baba*); Baba No. AM 19. Paratopotypes, 2 males. Paratype, male, Nikko, Shimotsuke, June 6, 1931 (*M. Ueno*).

Distinguished from typical *confluens* Alexander by the coloration and by the structure of the male hypopygium.

37 (30). *PEDICIA (TRICYPHONA) KIRISHIMENSIS* (Alexander). Plate 2, fig. 8.

Tricyphona kirishimensis ALEXANDER, Philip. Jour. Sci. 35 (1928) 474-475, pl. 1, fig. 6 (venation).

The type, a male, was from Mount Kirishima, Kyushu, taken June 9, 1926, by Issiki. Known also from Shikoku.

SETTSU: Ushitaki, Osaka, April 27, 1930 (*Teranishi*).

HOKI: Mount Daisen, altitude 1,200 meters, June 7, 1930 (*Hibi*).

The wings are quite unpatterned except for the weakly and uniformly darkened cell C; cell Sc and the stigma paler brownish yellow. Venation: Position of r-m variable, from slightly before fork of Rs to connecting with R₄₊₅ beyond its origin; m-cu at or beyond fork of M, in the latter case to more than one-half its own length.

Male hypopygium (Plate 2, fig. 8) with the tergite, *t*, large, narrowed posteriorly, terminating in a large transverse cushion, densely setuliferous, with fewer scattered pale setæ. Basistyle, *b*, with a very low lobe on face; interbase a very long needlelike spine from an enlarged base. Dististyle, *d*, with two elongate lobes, both obtuse at tips, the inner one shorter and a little stouter, with a few long setæ at tip; outer apical angle produced into a low setuliferous lobe, not spinelike as in confluens. Phallosome with both the ædeagus and gonapophyses long and conspicuous, tips broad and truncated.

38 (299). *PEDICIA (TRICYPHONA) NORIKURÆ* sp. nov.

Plate 2, fig. 9.

Generally similar to *seticauda* Alexander, differing especially in the structure of the male hypopygium (Plate 2, fig. 9). Tergite large, narrowed outwardly, the cephalic half with two large lobes that are directed dorsad (on slide mounts the lobes are directed either cephalad or caudad, as figured); lobes with abundant long yellow setæ. Basistyle, *b*, provided with abundant long setæ; interbase very large, shaped about as in figure; the constriction or flange before the apex, as shown, may be caused by pressure of the coverslip. Dististyle, *d*, with outer lobe low and obtuse, the apex with abundant short blackened pegs or spines; inner style suboval, apex obtuse, with several relatively long slender setæ.

Habitat.—Japan (Honshu).

Holotype, male, on slide, Norikuradake, Shinano, July 27, 1930 (*K. Kamiya*). Allotopotype, female, July 22, 1929 (*M. Ueno*). Paratopotype, male, July 26, 1929 (*Jiro Machida*). Paratype, male, "Northern Alps," without more exact data, August 21, 1934 (*S. Kariya*).

I have had this fly in my collection for several years while awaiting better preserved material. The species is allied to *Pedicia (Tricyphona) seticauda* (Alexander) but has the hypopygial details, particularly the tergite and dististyle, quite distinct.

- 39 (32). **PEDICIA (TRICYPHONA) SETICAUDA** (Alexander). Plate 2, fig. 10.
Tricyphona seticauda ALEXANDER, Ann. and Mag. Nat. Hist. (9)
15 (1925) 77-78.

The type was from Lake Ozenuma, on the boundary between Iwashiro and Kotsuke, altitude 5,460 feet, taken July 26, 1923, by Esaki. Also known from Hokkaido and Shikoku.

AKI: Kure, altitude 400 meters, October 16, 1955 (*Baba*); Baba No. SD 23.

ECHIGO: Kurokawa, September 30, 1954, (*Baba*) Baba No. 293. Mount Amakazari, altitude 600 meters, June 26, 1955 (*Baba*); Baba No. AM 18.

Male hypopygium (Plate 2, fig. 10) with the tergite narrowed outwardly, the posterior border subtruncate or with a very shallow emargination; dorsal surface on either side of midline with very large stout erect lobes that bear brushes of long yellow setæ (in slide mounts, lobes generally directed caudad, as shown). Basistyle, *b*, with mesal face provided with longer and stouter setæ; interbase a powerful rod, stouter than in some related species, the outer part or head narrowed to a point. Dististyle, *d*, with the outer lobe flat, apex set with abundant small blackened spicules; inner style an almost circular disk, the outer half with several small but conspicuous setæ.

- 40 (300). **PEDICIA (TRICYPHONA) SETIPENNIS** (Alexander.). Plate 2, fig. 11.
Tricyphona setipennis ALEXANDER, Philip. Jour. Sci. 44 (1931) 355,
pl. 1, fig. 11 (venation).

The type was from Norikuradake, Shinano, taken July 26, 1929, by Machida.

The species is most readily told from other regional members of the *occulta* group by the presence of conspicuous macrotrichia in the outer ends of cells R_2 to 2nd M_2 of the wings, stronger and more conspicuous in the radial field. Male hypopygium (Plate 2, fig. 11) distinctive in the very large simple tergite, without dorsal lobes as in allied forms. Ninth tergite narrowed posteriorly, the tip subtruncate to very shallowly emarginate, the outer half of both the upper and lower surface with abundant long yellow setæ. Basistyle, *b*, much smaller than the tergite, its mesal part provided with long setæ; interbase stout. Dististyle, *d*, with the outer part very obtuse, the apex with abundant small blackened pegs; inner or beak portion suboval, narrowed to the obtuse tip, near apex with several long setæ.

41 (301). *PEDICIA (TRICYPHONA) VETUSTA* (Alexander). Plate 2, fig. 12.

Tricyphona vetusta ALEXANDER, Can. Ent. 46 (1913) 320-321, pl. 3, fig. 5 (wing).

The type was from the vicinity of Tokyo, taken April 25, 1912 by Kuwana and assistants. Also known from Shikoku.

HIDA: Gifu, October 10, 1920, November 5, 1921 (*Takeuchi*); April 29, 1932 (*Kariya*). Mount Kinkwa, May 6 to 11, 1932 (*Kariya*).

SETTSU: Osaka, November 27, 1914 (*Nohira*).

MUSASHI: Tokyo, June 25, 1912.

Male hypopygium (Plate 2, fig. 12) with the tergite large, the central area of the posterior border produced caudad, its outer margin truncate; dorsal surface back from apex with two small erect darkened lobes provided with small weak setæ (lobes on slide mounts generally bent caudad by pressure); vestiture of remainder of tergite weak. Basistyle, *b*, small; interbase pale, terminating in a cultrate beak. Dististyle, *d*, with the outer lobe relatively small, the spicules small; inner lobe or beak with a row of unusually long setæ.

In addition to the species of the *occulta* group discussed at this time, two additional forms have been treated in earlier parts of this series of reports, *Pedicia (Tricyphona) fimbriatula* Alexander, of Shikoku (Part I, pp. 59-61) and *P. (T.) tenuiloba* Alexander, of Honshu (Part V).

42 (302). *PEDICIA (TRICYPHONA) DIAPHANOIDES* Alexander. Plate 1, fig. 6; Plate 2, fig. 13.

Pedicia (Tricyphona) diaphanoides ALEXANDER, Philip. Jour. Sci. 67 (1938) 150-151, pl. 1, fig. 15 (venation).

The types were from Ompo, North Korea, taken in May, 1937 and 1938 by Yankovsky.

Shinano: Ichinomata—Goya, Mount Jonen, 1,600 to 2,857 meters, July 27, 1951 (*Hiroshi Inoue*).

Wings (Plate 1, fig. 6) in cases with crossvein *m* weakly preserved, closing cell 1st *M*₂, in these cases cell *M*₁ a little longer than its petiole.

Male hypopygium (Plate 2, fig. 13) with the tergite large, its posterior border broadly emarginate, the lateral lobes large; margin fringed with delicate setæ; one ventral face near cephalic-lateral angle with a strong spine directed chiefly caudad. Basistyle, *b*, with the larger apical lobe suboval, with abundant long black spinelike setæ; smaller lobe provided with very few similar setæ; interbase a pale elongate flattened blade. Dististyle, *d*, a long compressed blade, the apex obtuse; surface of

outer part with rather numerous slender setæ. Phallosome, *p*, with the ædeagus terminating in two small points; outer ends of the gonapophyses very narrow.

NIPPONOMYIA Alexander

Nipponomyia ALEXANDER, Insec. Inscit. Menst. 12 (1924) 158-159.

Nipponomyia is best distinguished from *Pedicia* (*Tricyphona*) by the glabrous eyes, the distinctive wing pattern, venation, and basic structure of the male hypopygium. There are about a dozen known species, ranging from Japan through Formosa and eastern China to Borneo, Sumatra, and Assam.

The three local species may be separated by the accompanying key.

1. Costal cell of wings without transverse black dashes; crossvein *m* oblique; male hypopygium with three spines on dististyle.

trispinosa (Alexander)

Costal cell of wings with numerous transverse darkened lines or dashes; male hypopygium with the spines of the dististyle more numerous.

2. Spines of dististyle five in number; cell 1st *M*₂ closed.

pentacantha sp. nov.

Spines of dististyle small but numerous, approximately twelve in number; cell 1st *M*₂ usually open by the atrophy of *m*.

kuwanai (Alexander)

The homologies of certain parts of the male hypopygium remain somewhat in question. I have interpreted the entire outer end of the style as representing a single complex dististyle, with no outer lobe of the basistyle being present, as is the case in many *Pediciini*. However, in this tribe it is sometimes difficult to decide what is dististyle and what basistyle and the interpretation given here may be questioned. In this same connection, see also Edwards.³

43 (303). *NIPPONOMYIA KUWANAI* (Alexander).

Plate 3, fig. 16.

Tricyphona kuwanai ALEXANDER, Can. Ent. 45 (1913) 318-319, pl. 3, fig. 6 (wing).

Nipponomyia kuwanai ALEXANDER, Insec. Inscit. Menst. 12 (1924) 158-159.

Nipponomyia kuwanai ESAKI, ET AL., Icon. Insect. Japon. Ed. 2 (1950) 1521, fig.

The type, a female, was from Tokyo, taken May 7, 1912, by the late Dr. Shinkay Inokichi Kuwana, for whom it was named.

MUSASHI: Tokyo, April 7, 1919 (*Takahashi*); Meguro, April 15, 1919 (*Takahashi*).

³ Edwards, F. W. Soc. British Ent. Trans. 5 (1938) 52.

RIKUCHIU: Iwate-gun, altitude 3,000 feet, June 28, 1935 (H. Yamamoto).

Male hypopygium (Plate 3, fig. 16) with the basistyle, *b*, stout, the setæ unusually long and abundant; interbase extended into a long point, setæ restricted to its base. Dististyle, *d*, including a low oval outer cushion, provided with abundant slender spines and an inner compressed rostral portion, the beak slender, at its base with a compact group of about twelve strong black spines. Phallosome with ædeagus narrowly obtuse at tip.

44 (304). *NIPPONOMYIA PENTACANTHA* sp. nov.

Plate 3, figs. 14, 17.

Generally as in *kuwanai* in the coloration of the body and wings; cell 1st M_2 closed, *m* transverse; male hypopygium with the interbase dilated on outer half; dististyle with five strong spines at base of beak.

Male.—Length, about 12 to 13 millimeters; wing, 10 to 11.5; antenna, about 1.5 to 1.6.

Rostrum testaceous; palpi brownish black. Antennæ 14-segmented; scape light brown, pedicel and flagellum yellow; flagellar segments suboval, becoming more elongate outwardly, terminal segment long; verticils unilaterally distributed, one major one on each segment. Head obscure brownish yellow behind, the anterior vertex and orbits weakly infuscated.

Pronotum light yellow. Mesonotal præscutum fulvous, paling to yellow on sides, with a semicircular to subtransverse row of oval black spots about one-third the length from suture; posterior sclerites of notum yellow, the scutum with three black spots, including the cephalic part of each lobe near the suture and a triangular spot at the point of the suture; a further scarcely evident black spot on parascutella. Pleura yellow. Halteres pale yellow throughout. Legs with the femora yellow, the remainder paler yellow, the tips of the tibiæ narrowly dark brown, the three proximal tarsal segments narrowly infuscated; outer two tarsal segments uniformly brownish black. Wings (Plate 3, fig. 14) subhyaline, the costal cell with a series of transverse black dashes, as in *kuwanai*; immediately behind this border with a broad yellow longitudinal stripe that extends virtually to the wing tip, in cells *R* and R_1 bordered behind by a broken brownish black line, in the outer radial field margined above and below by light brown; a further oblique light brown line from near the tip of Sc_1 across the fork of *Rs* to the outer end of cell 1st M_2 , the cord more narrowly bordered

by pale brown; a narrow black dash in cell M at base, adjoining vein M; vein Cu bordered by brighter yellow; veins yellow, M and Rs darker. Venation: r-m some distance before fork of Rs; R_{2+3} nearly perpendicular at origin, lying close to R_1 ; cell R_4 sessile; cell 1st M_2 closed, m transverse, connecting M_2 beyond its base with M_3 beyond midlength; m-cu close to the fork of M.

Abdomen yellow, the tergites with a narrow broken pale brown central stripe; tergites with further conspicuous black lateral lines, sternites with comparable sublateral dashes; hypopygium yellow. Male hypopygium (Plate 3, fig. 17) with the basistyle, *b*, stout; interbase short, its outer half dilated, terminating in an acute point, setæ confined to basal part. Dististyle, *d*, occupying the entire outer end of style, the outer part a small oval lobe set with abundant slender spinlike setæ, the inner portion extended into a slender beak, at its base with a compact group of five large spines. Phallosome small.

Habitat.—Japan (Honshu).

Holotype, male, Mount Amakazari, Echigo, altitude 600 meters, June 25, 1955 (*Baba*); Baba No. AM 28. Paratopotype, male, altitude 300 meters, June 26, 1955 (*Baba*).

Readily told from the most similar regional species, *Nipponomyia kuwanai* (Alexander) by the structure of the male hypopygium.

45 (243). *NIPPONOMYIA TRISPINOSA* (Alexander),

Plate 3, fig. 18.

Nipponomyia trispinosa (Alexander), Philip. Jour. Sci. 83 (1954) 285-286 (latest references).

The type was from "Honshu," without further data (*Akio Nohira*); Nohira No. 28.

AKI: Kure, altitude 400 meters, October 16, 1955 (*Baba*); Baba No. SD 17.

HIDA: Gifu September 15, 1921 (*Takeuchi*); November 15, 1931 (*Kariya*).

OMI: Otsu, Lake Biwa, October 9, 1905, swarming near ground (*Annandale*).

Male hypopygium (Plate 3, fig. 18) with the interbase, *i*, of the basistyle, *b*, dilated beyond base, thence narrowed into a sharp spine, the surface of the basal and central parts with several setæ, some of unusual length. Dististyle, *d*, with the body produced, the face with numerous long blackened spinules.

that extend far cephalad along the style, near apex of lobe with numerous long setæ; rostrum slender, spines three, very strong. Phallosome with the gonapophysis elongate, subtending the ædeagus which is extended into a long aciculate point.

HETERANGÆUS Alexander

Heterangæus ALEXANDER, Ann. and Mag. Nat. Hist. (9) 15 (1925) 78-79.

Antennæ 12- or 13-segmented. Wings commonly cross-banded with brown. Venation: Rs relatively long, angulated and spurred at origin; R_{2+3} perpendicular at base, with a spur jutting backward into cell R_1 ; R_2 far distad, R_{1+2} correspondingly short; cell 1st M_2 closed; position of m variable, in cases connecting M_2 with M_3 , in other instances lying more proximad; supernumerary crossveins in cells R_3 , R_4 , M_1 and M, the last approximately opposite the origin of Rs (Plate 3, fig. 15). Male hypopygium (Plate 3, fig. 19) with the dististyle, *d*, terminating in a single powerful erect spine. Ædeagus usually short, terminating in a capitate head, in *japonicus* elongate, extended into a needlelike spine.

There evidently is great variation in the relative positions of the supernumerary crossveins in the different cells and their location can be used as a taxonomic character only within limits.

The known species of *Heterangæus* may be told by the following key:

1. Legs brownish black, the femoral bases restrictedly yellowed.
spectabilis (Alexander)
 Legs chiefly yellow, the tips of the femora and tibiæ dark brown to brownish black 2
2. Cell C of wings uniformly darkened 3
 Cell C chiefly pale, variegated with dark areas at base, apex and above the origin of Rs 6
3. Darkened pattern of the wing disk faint to subobsolete, at first sight the wing appearing to be virtually unpatterned; a dusky longitudinal streak over much of the length of cell R *esakii* (Alexander)
 Darkened pattern of the wing disk conspicuous, appearing as cross-bands and seams over the veins and deflections; no dark longitudinal streak in cell R 4
4. Size larger (wing 10 millimeters or more); darkened wing bands commonly with the centers only a little paler than the margins; bands broad, the interspaces correspondingly reduced; darkened spots in cell Cu more or less confluent, in cases forming larger areas.
laticinctus Alexander
 Size smaller (wing usually less than 9 millimeters); darkened wing bands pale with brown margins; bands narrow, the interspaces correspondingly wide; darkened spots in cell Cu isolated. (*japonicus*) 5

5. Darkened wing bands narrower than the pale interspaces.
 crenate *japonicus japonicus* Alexander
 Darkened wing bands broader, the brown margins less evident; posterior border, especially in the cubital and anal fields, strongly crenate *japonicus crenatus* Alexander
6. Wing pattern very pale, cell Cu_2 chiefly clear *pallidellus* Alexander
 Wing pattern dark and clearly defined, including areas in cell Cu_2 , these reduced in *gloriosus kusunokii*. (*gloriosus*) 7
7. Cell Cu_2 with conspicuous darkenings, most of which completely traverse the cell Sc_2 narrow vein Sc . transverse.
 gloriosus gloriosus (Alexander)
 Cell Cu_2 with dark markings reduced, not completely traversing the cell; cell Sc broad, vein Sc_2 oblique. (Shikoku).
 gloriosus kusunokii Alexander

46 (305). **HETERANGÆUS ESAKII** Alexander.

Heterangæus esakii ALEXANDER, Ann. Mag. Nat. Hist. (9) 15 (1925) 79-80.

Types from Lake Ozenuma, on boundary between Iwashiro and Kotsuke, altitude 5,460 feet, taken July 28, 1923, by Teiso Esaki.

47 (179). **HETERANGÆUS GLORIOSUS** (Alexander).

Polyangæus gloriosus ALEXANDER, Philip. Jour. Sci. 24 (1924) 569-571.

The types were from Shimizu, Karafuto, taken July 27, 1922, by Esaki.

ECHIGO: Mount Amakazari, altitude 900 meters, June 25, 1955 (*Baba*); *Baba* No. AM 30.

I am referring this specimen to *gloriosus* with a slight question and the discovery of more material, especially the male, may make it desirable to propose a new subspecies or species. The wings are narrow, with the pale interspaces of both cells C and Sc speckled with dark brown.

48 (34). **HETERANGÆUS JAPONICUS** (Alexander).

Polyangæus japonicus ALEXANDER, Ent. Soc. America Ann. 12 (1919) 342.

The type was from the mountains of Musashi (Saitama), where it was taken May 31, 1919 by Takahashi.

RIKUCHIU: Iwate-gun, altitude 3,000 feet, May 17, 1935 (*Yamamoto*).

48a (34a). **HETERANGÆUS JAPONICUS CRENATUS** Alexander.

Heterangæus japonicus crenatus ALEXANDER, Philip. Jour. Sci. 51 (1933) 541.

The type was from Mount Ohdai, Yamato, taken June 5, 1930, by S. Sakaguchi.

49 (306). *HETERANGÆUS LATICINCTUS* Alexander.

Plate 3, fig. 19.

Heterangæus laticinctus ALEXANDER, Philip. Jour. Sci. 44 (1931) 355-357.

The types were from Hirayu, Shinano, taken July 26 to 27, 1929, by Jiro Machida.

HIDA: Mount Ontake, altitude 5,850 feet, July 6 to 10, 1934 (*H. Ise*).

MINO: Gifu, June 6, 1931 (*S. Sawada*); Mount Kinkwa, Gifu, May 11 to 23, 1932, April 28, 1933 (*Kariya*).

Male hypopygium (Plate 3, fig. 19) with the tergite transverse, the posterior border scarcely produced, the central area truncate or very insensibly trilobed, with abundant microscopic setulæ. Basistyle, *b*, produced into a very stout apical lobe or cushion, provided with numerous slender spinoid setæ; interbase, *i*, a long narrow blade, the tip acute. Dististyle, *d*, long and narrow, curved at base, tip obtuse; before apex with a single elongate spinoid seta; lower margin of style near tip with about eight very long pale setæ. Phallosome, *p*, with the gonapophyses appearing as pale setuliferous cushions; ædeagus short, the apex capitate.

As is the case with various other Pediciine groups, there is a question as to whether the spiniferous terminal lobe of the basistyle is not actually a dististyle, as so interpreted under the discussion of *Nipponomyia*, ante.

50 (307). *HETERANGÆUS PALLIDELLUS* Alexander.

Heterangæus pallidellus ALEXANDER, Philip. Jour. Sci. 51 (1933) 540-541, pl. 1, fig. 21 (venation).

The types were from Mount Kurobegoro, Etchu, taken in subalpine meadows at an altitude of 7,800 feet, August 8, 1931, by Imanishi.

51 (308). *HETERANGÆUS SPECTABILIS* Alexander.

Heterangæus spectabilis ALEXANDER, Ann. Mag. Nat. Hist. (9) 15 (1925) 80-81.

The type was from Hinoëmata, Iwashiro, altitude 4,000 feet, taken on July 24, 1923, by Esaki.

DICRANOTA Zetterstedt

Dicranota ZETTERSTEDT, Ins. Lapponica, Dipt. (1838) 851.

The genus *Dicranota* includes the small and obscure members of the Pediciini, represented by numerous species in Japan.

In another report,⁴ I have attempted to separate out various subgeneric groups within the genus but these are based chiefly on weak and insufficient characters and are difficult to maintain except for convenience of treatment of the abundant forms.

52 (309). **DICRANOTA (DICRANOTA) NIPPOALPINA** Alexander.

Dicranota (Dicranota) nippoalpina ALEXANDER, Philip. Jour. Sci. 50 (1933) 147-148, pl. 1, fig. 14 (venation), pl. 3, fig. 42 (♂ hypopygium).

The type was from Shiroumadake, Shinano, taken August 8, 1931, by Machida.

53 (310). **DICRANOTA (DICRANOTA) SICARIA** Alexander.

Plate 4, fig. 29.

Dicranota (Dicranota) sicaria ALEXANDER, Ent. Soc. America Ann. 40 (1947) 354.

The types were from Hamasaka, Harima, taken April 1, 1931, by J. Okada.

Male hypopygium (Plate 4, fig. 29) with the tergite large, the median area of the posterior border only vaguely produced, with weak setæ; lateral tergal arms large and conspicuous, pale, dilated and more or less recurved at tip. Basistyle, *b*, with two apical lobes, one longer and more slender, both with normal setæ only; interbase appearing as a flattened blade, at apex produced into a long straight spinelike blade. Dististyle, *d*, a flattened oval blade, the tip obtusely rounded, with several strong setæ. Phallosome, *p*, with the gonapophyses divergent.

54 (311). **DICRANOTA (RHAPHIDOLABIS) ANGULATA** Alexander.

Dicranota (Rhaphidolabis) angulata ALEXANDER, Philip. Jour. Sci. 60 (1936) 182-183, pl. 1, fig. 9 (venation).

The type, a female, was from Iwate-gun, Rikuchiu, altitude 3,000 feet, taken June 9, 1935, by Yamamoto. The male sex is still unknown.

55 (312). **DICRANOTA (RHAPHIDOLABIS) BABAI**, sp. nov.

Plate 4, figs. 20, 23.

General coloration dark brown, the præscutum with three brownish black stripes; antennæ 17-segmented, relatively short; wings relatively narrow, tinged with dusky; Rs unusually long, cell R_3 sessile; m-cu short, at near midlength of M_{3+4} ; male hypopygium with the median region of tergite produced into a depressed plate, its posterior border emarginate; lateral tergal arms powerful; basistyle elongate, with a small apical lobe; dististyle much longer.

⁴ Alexander, C. P. Entomological results from the Swedish Expedition 1934 to Burma and British India. Diptera: Tipulidæ-Pediciini. Arkiv för Zoologi 42 A 2 (1949) 17-18.

Male.—Length, about 5.5 millimeters; wing, 6.1; antenna, about 0.9.

Rostrum and palpi brownish black. Antennæ short, 17-segmented, black throughout; flagellar segments oval, becoming progressively smaller outwardly. Head dark gray.

Pronotum dark brown. Mesonotal præscutum brown, with three brownish black stripes, the sides more pruinose; scutal lobes dark, scutellum testaceous brown; postnotum slightly darker. Pleura uniformly brown, unpatterned; dorsopleural membrane dark. Halteres with stem dirty white, knob infuscated. Legs with the coxæ and trochanters light brown; remainder of legs somewhat darker brown. Wings (Plate 4, fig. 20) relatively narrow, tinged with dusky; stigmal region large, slightly more darkened; veins brown, those at base and in proximal part of costal region paler. Venation: Rs unusually long; R_2 about twice R_{1+2} ; cell R_3 sessile, cell R_4 virtually so, there being a very short element R_{4+5} ; m-cu short, at near midlength of M_{3+4} .

Abdomen, including hypopygium, dark brown. Male hypopygium (Plate 4, fig. 23) very distinctive. Ninth tergite, *t*, transverse, the median area produced caudad into a broad depressed plate, its posterior border with a broad U-shaped notch, lobes narrower; surface with abundant setulæ; lateral tergal arms strongly developed into sinuous spines that gradually narrow to acute points. Basistyle, *b*, elongate, at tip with a small fleshy lobe, with several long setæ; interbase a powerful arm of approximately the same size and shape as the lateral tergal spines; basad of the interbase, on mesal face, with a compact group of five or six long setæ; what appears to be a still further outgrowth of the basistyle, at proximal end (not shown in figure) appears as an elongate densely setuliferous lobe lying beneath the tergal plate. What is interpreted as being the dististyle, *d*, is a long slender structure, fully three times as long as the lobe of the basistyle, at near midlength with a lateral lobe that is provided with several strong setæ, the outer extended part gradually narrowed to the obtuse tip, bearing other smaller setæ. What appears to represent the phallosome (not shown in figure) appears as a central triangular plate, the outer arms extended laterad, their tips obtuse. As in other Pediciine forms, it seems difficult to decide which of the outer extensions of the basistyle are actual lobes of the same or represent dististyles.

Habitat.—Japan (Honshu).

Holotype, male, Kure, Aki, altitude 400 meters, October 16, 1955 (*Baba*); *Baba* No. SD 19.

I take unusual pleasure in dedicating this distinct fly to Dr. Kintaro Baba. It is very different from other known species of the subgenus that have cell R_3 of the wings sessile in the great number of antennal segments and in the very peculiar structure of the male hypopygium. *Dicranota* (*Rhaphidolabis*) *polymera* Alexander similarly has the antennæ 17-segmented but in all other respects is a very different fly.

56 (313). *DICRANOTA* (*RHAPHIDOLABIS*) *BASISTYLATA* sp. nov. Plate 4, figs. 21, 24.

Allied to *ontakensis* and *trilobulata*; general coloration gray, the præscutum with three brown stripes, the median one more or less split by a pale central vitta; antennæ black, flagellar segments subglobular to short-oval; legs obscure yellow to light brown; wings milky white, stigma slightly darker, veins pale brown; R_{2+3+4} distinct; male hypopygium with the tergite terminating in a plate that divides into two fingerlike lobes; basistyle produced dorsad into a large clavate lobe and ventrad into an even larger structure, both provided with long white setæ; interbase with two unequal spines; dististyle a pale boomerang-shaped blade.

Male.—Length, about 5 to 5.2 millimeters; wing, 6.2 to 6.5; antenna, about 1.0 to 1.1.

Female.—Length, about 5.5 millimeters; wing, 7.

Rostrum and palpi black. Antennæ short, black throughout; flagellar segments subglobular to short-oval, longer than the verticils. Head dark gray.

Pronotum gray. Mesonotal præscutum dark gray, with three brown stripes, the broad central vitta very narrowly and indistinctly split by a capillary pale line, in cases showing four distinct stripes; posterior sclerites of notum gray, the centers of the scutal lobes somewhat darker. Pleura uniformly gray. Halteres with stem whitened, knob very weakly darkened. Legs with the coxæ gray; trochanters obscure yellow; femora obscure yellow, the tips slightly darkened; tibiæ and tarsi light brown. Wings (Plate 4, fig. 21) milky white, the prearcular and costal regions a trifle more yellowed; stigma very faintly more darkened, scarcely evident; veins light brown. Venation: R_s short, strongly arcuated to weakly angulated at near mid-length, R_{2+3+4} distinct; R_{1+2} shorter than R_2 , the latter very

slightly oblique; m-cu about one-third to one-half its length beyond the fork of M.

Abdomen dark gray, hypopygium brownish gray. Male hypopygium (Plate 4, fig. 24) very distinctive, especially because of the major lobes of the basistyle, *b*. Ninth tergite, *t*, produced into a more or less erect plate that divides into two fingerlike lobes separated by a broad notch. Basistyle, *b*, stout, very complex; outer apical angle produced into a large clavate lobe, densely provided with long pale setæ; on mesal face at near midlength with a small protuberance that bears numerous slender dark-colored spines or points; on mesal face of body of style near outer end with a small oval lobe, provided with abundant setæ; ventral part of style produced into large conspicuous lobes or flaps, directed chiefly ventrad, provided with abundant long white setæ that become smaller basally and extend up the face of the style to the mesal lobe above described; interbase very conspicuous, broad-based, forking into two unequal spines. Dististyle, *d*, shorter and narrower than the outer lobe of basistyle, appearing as a flattened blade, somewhat boomerang-shaped, gradually narrowed and curved to the obtuse tip; surface with microscopic pale punctures, on the lower margin at near midlength with long pale setæ. Phallosome, *p*, with the slender ædeagus darkened; gonapophyses shorter and broader.

Habitat.—Japan (Honshu).

Holotype, male, Kurokawa, Echigo, April 26, 1955 (*Baba*); *Baba* No. 413. Allotopotype, female. Paratopotypes, 3 males, 1 female, April 20 to 29, 1955 (*Baba*).

This conspicuous fly is readily told from *Dicranota* (*Rhaphidolabis*) *ontakensis* Alexander and *D. (R) trilobulata* sp. nov., by the structure of the male hypopygium, especially the large ventral lobes of the basistyle which differentiate the fly from all other known species of the subgenus.

57 (314). DICRANOTA (RHAPHIDOLABIS) MACRACANTHA Alexander. Plate 4, fig. 25.

Dicranota (*Rhaphidolabis*) *macracantha* ALEXANDER, Ent. Soc. America Ann. 40 (1947) 354-355.

The type was from Kamikochi, Shinano, altitude 5,000 feet, taken August 18 to 21, 1934, by Kariya.

Male hypopygium (Plate 4, fig. 25) with the tergite, *t*, transverse, the median area of posterior border produced into a broad lobe, its apex truncate, surface with abundant long setæ that are directed caudad; lateral tergal arms very long and

powerful, fully three times as long as the lobe, appearing as sinuous yellow blades that narrow to the acute tips. Basistyle, *b*, terminating in two lobes, a short outer one and a longer clavate lobe, both with relatively numerous blackened spinoid setæ at and near tips; interbase, *i*, a powerful flattened glabrous yellow blade, narrowed into a long acute spine that is bent at about a 45° angle to the axis; immediately back from the point, both above and below, with a low flange. Dististyle, *d*, about as long as the longer lobe of the basistyle narrowed gradually to the obtuse tip, with a few long strong retrorse setæ near apex; on face of style at base with a fold or flange (in microscope slide). Phallosome, *p*, with ædeagus darkened, constricted near outer end to form an oval head; gonapophysis dilated into a broad obtuse hyaline blade.

58 (315). DICRANOTA (RHAPHIDOLABIS) ONTAKENSIS Alexander. Plate 4, fig. 26.

Dicranota (Rhaphidolabis) ontakensis ALEXANDER, Ent. Soc. America Ann. 40 (1947) 355-356.

The types were from Mount Ontake, Hida, altitude 5,850 feet, taken July 6 to 10, 1934, by H. Ise.

Ugo: Kuroyu, altitude 850 meters, June 13, 1951 (*Issiki-Ito*).

Male hypopygium (Plate 4, fig. 26) with the tergite, *t*, large, the median area of the posterior border produced caudad into a depressed blade that terminates in two broad lobes, separated by a narrower notch, each lobe with abundant setæ. Basistyle, *b*, with outer apical lobe very stout, capitate, the stem short, head very large, obtusely rounded, set with abundant peglike spines or spicules, these becoming more hairlike toward base; interbase, *i*, a conspicuous bispinous plate, the spines slightly unequal; mesal face of style beyond the interbase with a group of very long setæ. Dististyle, *d*, slender, slightly widened outwardly, the tip obtuse, with a very few strong subapical setæ.

59 (316). DICRANOTA (RHAPHIDOLABIS) PLATYMERIA Alexander.

Dicranota (Rhaphidolabis) platymera ALEXANDER, Philip. Jour. Sci. 53 (1934) 279-280, pl. 1, fig. 8 (venation), pl. 2, fig. 30 (♂ hypopygium).

The types were from Yumoto, Shimotsuke, altitude 4,850 feet, taken June 20, 1932, by Issiki.

60 (317). DICRANOTA (RHAPHIDOLABIS) PROFUNDA Alexander. Plate 4, fig. 28.

Dicranota (Dicranota) profunda ALEXANDER, Ent. Soc. America Ann. 43 (1950) 425-426.

The types were from Funakosi, Rikuchiu, taken May 21, 1947, by Yamamoto.

ECHIGO: Kitakanbara, Sugatani, altitude 300 meters, May 8, 1955 (*Hiroshi Koike*); Baba No. 465. Kurokawa, May 5, 1954 (*Baba*); Baba No. 224.

Although the type material showed a supernumerary cross-vein in cell R_1 of the wings, as in the subgenus *Dicranota*, additional specimens lack this vein and I am now referring the fly to the subgenus *Rhaphidolabis*.

Male hypopygium (Plate 4, fig. 28) with the tergite large, the posterior border profoundly divided medially, the parts thus formed further produced caudad into a narrow submedian lobe, tipped with short bristles, in cases with a single marginal seta on either side. Basistyle, *b*, with the small dorsal lobe tipped with a few short spinoid setæ; outer lobe relatively long, cylindrical, with numerous setæ, those on outer third stouter; interbase very large and flattened, the outer margin microscopically serrulate. Dististyle, *d*, subequal in length to the outer lobe of the basistyle, expanded at base, the outer two-thirds narrowed, tip obtuse; inner margin of basal half with abundant setæ.

61 (318). *DICRANOTA (RHAPHIDOLABIS) SPINA* Alexander.

Dicranota (Rhaphidolabis) spina ALEXANDER, Philip. Jour. Sci. 51 (1933) 398-399, pl. 1, fig. 17 (venation), pl. 4, fig. 48 (♂ hypopygium).

The holotype male was from Mount Kurobegoro, Etchu, altitude 7,800 feet, taken in a subalpine meadow on August 13, 1931, by Imanishi. Known also from Hokkaido.

ECHIGO: Mount Zaô, Iwafune, May 3, 1955 (*Baba*); Baba No. 432 B.

62 (319). *DICRANOTA (RHAPHIDOLABIS) TRILOBULATA* sp. nov. Plate 4, figs. 22, 27.

Allied to *ontakensis*; general coloration of thorax light brown, heavily pruinose, mesonotum unpatterned; legs light brown; wings milky white, unpatterned, veins pale; R_{2+3+4} long; male hypopygium with the tergite produced caudad into a narrow lobe, its tip with three subequal lobules, all with numerous short setæ; outer lobe of basistyle stout, tip obtuse, densely spiculose; interbase bispinous; dististyle long and slender, nearly parallel-sided, near the tip with a few long setæ.

Male.—Length, about 5.5 millimeters; wing, 6; antenna, about 1.2.

Rostrum brown; palpi brownish black. Antennæ black; flagellar segments oval, a little longer than their verticils. Head gray.

Thoracic dorsum light brown, heavily pruinose, without pattern; pleura and pleurotergite more yellowed. Halteres yellow, including the large knob. Legs with the coxæ and trochanters yellow; remainder of legs light brown, the femoral bases a little more yellowed; outer tarsal segments darker. Wings (Plate 4, fig. 22) milky white, the prearcular and costal regions a trifle more yellowed, stigma barely indicated; veins poorly visible, very pale brown, yellowed in the brightened fields. Venation: Rs strongly arcuated to weakly angulated; R_{2+3+4} long; m-cu about three-fourths its length beyond the fork of M.

Abdomen medium brown, hypopygium, especially the styli, more brownish yellow. Male hypopygium (Plate 4, fig. 27) with the tergite transverse, the posterior border produced caudad into a narrow lobe that terminates in three subequal lobules, all with numerous short stout setæ. Basistyle, *b*, stout, the outer apical lobe short, a little dilated outwardly, apex very obtuse, with abundant short peglike spicules or spinoid setæ, these more elongate and hairlike nearer the base; interbase, *i*, a large bispinous plate, the lower spine a little larger than the outer one. Dististyle, *d*, long and slender, nearly parallel-sided, tip obtuse, just before apex with a few long retrorse setæ. Phallosome, *p*, small; ædeagus with tip obtuse.

Habitat.—Japan (Honshu).

Holotype, male, Kurokawa, Echigo, altitude 300 meters, June 11, 1955 (*Baba*); Baba No. 429.

The most similar species is *Dicranota* (*Rhaphidolabis*) *ontakensis* Alexander, which is most readily distinguished by the structure of the male hypopygium, particularly the tergite.

63 (320). DICRANOTA (RHAPHIDOLABIS) TUBERCULATA Alexander.

Dicranota (*Rhaphidolabis*) *tuberculata* ALEXANDER, Philip. Jour. Sci. 59 (1936) 241-242, pl. 1, fig. 13 (venation).

The types, represented by females, were from Kibune, Kyoto, Yamashiro, taken October 11, 1934, by Tokunaga. The male is still unknown.

ERIOPTERINI

CLADURA Osten Sacken

Cladura OSTEN SACKEN, Acad. Nat. Sci. Philadelphia Proc. (1859) 229.

Honshu is extremely rich in species in this interesting genus of autumnal crane-flies.

64 (321). *CLADURA (CLADURA) ALPICOLA* Alexander.

Cladura alpicola ALEXANDER, Philip. Jour. Sci. 40 (1929) 341-342, pl. 1, fig. 17 (venation), pl. 3, fig. 38 (♂ hypopygium).

Cladura (Cladura) alpicola ALEXANDER, Brooklyn Ent. Soc. Bull. 50 (1955) 18.

The types were from various stations in Shinano, taken by Machida on Tsubakuro, on August 23, 1925, and on Yarigatake, on August 24, 1925. Later records, similarly from Shinano, include Norikuradake, near Reisentoge, altitude 2,400 meters, September 8, 1951, and near Kwaigahara, altitude 2,600 meters, September 8, 1951, and Sirahoneonsen, altitude 1,500 meters, September 9, 1951, all taken by H. Hasegawa.

64a (321a). *CLADURA (CLADURA) ALPICOLA SETULILOBA* Alexander.

Cladura alpicola setuliloba ALEXANDER, Ent. Soc. America Ann. 40 (1947) 360.

The unique type male was from Mount Myotaka, taken August 1, 1923, by Fuzimatsu.

65 (322). *CLADURA (CLADURA) AUTUMNA* Alexander.

Plate 5, fig. 31.

Cladura autumnna ALEXANDER, Insec. Inscit. Menst. 7 (1919) 137.

Cladura autumnna ALEXANDER, Philip. Jour. Sci. 31 (1926) 370, pl. 2, fig. 9 (♂ hypopygium, gonapophysis).

The types were from Meguro, Tokyo, taken October 22, 1919, by Takahashi.

MINO: Gifu, October 7 to 10, 1920 (*K. Takeuchi*).

MUSASHI: Tokyo, October 31, 1929 (*B. Oda*).

SETTSU: Osaka, Kibune, October 16, 1934 (*Tokunaga*).

Male hypopygium (Plate 5, fig. 31) with the posterior border of the tergite narrowly notched, forming two low lobes, with a further lobule on ventral side cephalad of the outer one. Basistyle, *b*, stout, without lobes. Dististyle, *d*, terminal in position, simple, nearly as long as the basistyle, stout, the obtuse tip pendant; lower surface with abundant setæ, smaller but very dense at apex. Gonapophysis, *g*, appearing as a flattened blade, terminating in two weak teeth, with further irregular serrations on lateral margin; inner or upper edge with relatively sparse and weak setæ on outer half.

66 (323). *CLADURA (CLADURA) BABAI* Alexander.

Plate 5, fig. 32.

Cladura (Cladura) babai ALEXANDER, Brooklyn Ent. Soc. Bull. 50 (1955) 18-19.

The types were from Kurokawa, Echigo, taken October 15, 1953, by Kintaro Baba, with other material from Mount Chokai, Uzen, altitude 600 meters, October 17, 1950 (*Kotaro Shirahata*) and from Morioka, Rikuchiu, October 6, 1934 (*T. Kato*).

Male hypopygium (Plate 5, fig. 32) resembling that of *daimio*, differing in details. Tergal lobes, *t*, greatly expanded apically, subtrigonal in outline, with long lateral setæ. Basistyle, *b*, with the apical lobe narrowed gradually to the obtuse tip which bears a group of very long setæ; basal lobe unequally bilobulate, all with dense appressed setulæ. Dististyle, *d*, much stouter than the outer lobe of the basistyle, its apex strongly bilobulate. Gonapophysis, *g*, a powerful blade, strongly recurved into an acute spinous point. *Ædeagus* slender.

67 (324). *CLADURA (CLADURA) BIDENS* Alexander.

Plate 5, fig. 33.

Cladura bidens ALEXANDER, Philip. Jour. Sci. 31 (1926) 369-370, pl. 2, figs. 6, 8 (♂ hypopygium).

The types were from Sapporo, Hokkaido, taken October 6, 1923, by M. Hori.

ECHIGO: Kanno, Iwafune, October 7, 1954 (*Baba*); *Baba* No. 56.

Male hypopygium (Plate 5, fig. 33) generally as in *autumna*, differing especially in the structure of the gonapophysis. Tergite with the outer lobes low and obtuse, the cephalic pair longer, in cases conspicuously so. Basistyle and dististyle much as in *autumna*. Gonapophysis, *g*, appearing as a flattened blade that terminates in two long points; margins of blade without serrations, as in *autumna* and *serrimargo*.

68 (325). *CLADURA (CLADURA) BICORNUTA* Alexander.

Plate 5, fig. 34.

Cladura (Cladura) bicornuta ALEXANDER, Ent. Soc. America Ann. 48 (1955) 373.

The types were from various stations in Shinano, taken by H. Hasegawa-Sirahoneonsen, altitude 1,500 meters, September 9, 1951; near Reisen-goya, Mount Norikura, altitude 2,400 meters, September 8, 1951.

Male hypopygium (Plate 5, fig. 34) with the tergite, *t*, distinctive; posterior border with a central plate, its posterior border deeply U-shaped, the lateral arms narrow, obtuse at tips; lateral tergal plates adjoining the central one with a group of six or seven very long pale setæ; sides of lateral plate produced into a slender curved arm or horn. Basistyle, *b*, with the apical lobe about three-fourths as long as the dististyle, the tip obtuse, with long setæ; on mesal face of style with a slender curved lobe; basal lobe large, gradually narrowed to a subacute point, the enlarged cephalic part with a group of very long setæ. Dististyle, *d*, a simple rod, very gradually narrowed to the obtuse microscopically spiculose tip. Gonapophysis, *g*,

appearing as a flattened blade, about four-fifths as long as the ædeagus.

69 (326). *CLADURA (CLADURA) BREVIFILA* sp. nov. Plate 5, figs. 30, 35.

Allied to *telephallus*; male hypopygium of the same general structure, the details distinct; spinulose mesal lobe of basistyle more oval; ædeagus terminating in three shorter filaments, the longer one with a flange on its proximal two-thirds, the shorter paired filaments about two-thirds as long.

Male.—Length, about 6 to 6.5 millimeters; wing, 7 to 7.5.

Female.—Length, about 6.5 millimeters; wing, 7.

Rostrum yellow; palpi yellow basally, the terminal segment infuscated. Antennæ with scape and pedicel yellow, flagellum a little darker, especially at base. Head yellowish brown.

Thoracic dorsum fulvous yellow, unpatterned; posterior sclerites and pleura clearer yellow. Halteres with stem whitened, knob yellow. Legs with coxæ and trochanters light yellow; femora yellow, the tips narrowly brownish black; tibiæ brownish yellow, still more narrowly darkened at tips; tarsi passing into dark brown. Wings (Plate 5, fig. 30) subhyaline, the prearcular and costal fields a trifle more yellowed; stigma not indicated; veins pale brown, those in the prearcular and costal fields more yellowed. Venation: Sc_1 ending about opposite fork of R_{2+3+4} ; petiole of cell M_1 about twice m ; $m-cu$ at one-third to one-fourth the length of M_{3+4} .

Abdomen in male yellowish brown, with a narrow dark brown subterminal ring; in female, abdomen more uniformly dark brown; genital segment in both sexes yellowed. Male hypopygium (Plate 5, fig. 35) with the tergite, t , terminating in a simple depressed plate that is divided at apex into two obtuse lobes, surface of plate glabrous, except at base. Basistyle, b , with outer lobe shorter than the dististyle, elongate-subcylindrical, with conspicuous setæ, especially at and near tip; appendage of mesal face of style conspicuous, bilobed, the outer lobe oval, densely set with short spiculose points, inner lobe subequal in size, with coarse setæ. Dististyle, d , stout basally, the outer fourth narrowed and strongly recurved, the lower margin of the dilated base with long coarse setæ, the upper margin of the narrowed outer part with somewhat shorter bristles. Phallosome, p , including subcircular basal apophyses at base of a very large ædeagus that terminates in three short filaments, the longer one with a flange on the proximal two-thirds, the shorter paired filaments about two-thirds as long.

Habitat.—Japan (Honshu).

Holotype, male, Mount Tanigawad Kotsuke, altitude 800 meters, October 24, 1955 (*Baba*); Baba No. SD 38.

Allotopotype, female, pinned with type. Paratopotype, 1 male.

The most similar species is *Cladura* (*Cladura*) *telephallus* Alexander, which is told most readily by the structure of the male hypopygium, particularly the ædeagus.

70 (327). *CLADURA* (*CLADURA*) *DAIMIO* Alexander.

Plate 5, fig. 36.

Cladura daimio ALEXANDER, Ent. Soc. America Ann. 40 (1947) 359–360.

The type was from Morioka, Rikuchiu, taken October 6, 1934, by T. Kato.

Male hypopygium (Plate 5, fig. 36) much as in *babai*, differing in details. The tergal blades obtusely rounded at tips. Basistyle, *b*, with the proximal lobe large, the major inner part or lobule with abundant coarse setulæ, the outer arm or lobule a flattened oval blade that is nearly glabrous except for a few scattered setæ near apex. Dististyle, *d*, gradually dilated outwardly, broadest at near three-fourths the length. Phallosome, *p*, with the apophyses stout, curved, the tips obtuse; ædeagus much stouter than in *babai*, dilated shortly before the obtuse tip which has several pale circular punctures.

The hypopygium of the unique type specimen is mounted on a slide and is defective in certain regards. In particular, the gonapophyses, as shown, may be broken at their tips and not end abruptly as is shown. Except for the recurved pointed gonapophyses, the most decisive character for separating this fly from *babai* lie in the nature of the basal lobe of the basistyle and the structure of the ædeagus.

71 (328). *CLADURA* (*CLADURA*) *FUSCIVENA* Alexander.

Cladura (*Cladura*) *fuscivena* ALEXANDER, Ent. Soc. America Ann. 48 (1955) 372–373.

The unique type, a female, was from Sugadaira, Shinano, taken October 16, 1950, by H. Hasegawa.

72 (254). *CLADURA* (*CLADURA*) *HAKONENSIS* Alexander

Plate 5, fig. 37.

Cladura hakonensis ALEXANDER, Ent. Soc. America Ann. 40 (1947) 362–363.

The unique type female was from Gora, Hakone District, taken November 11, 1931 by Sawada.

SHINANO: Sugadaira, October 16, 1950 (*H. Hasegawa*).

KOTSUKE: Oze, Hatomachi Toge, September 22, 1950 (*Hasegawa*).

Male hypopygium (Plate 5, fig. 37) with the tergite produced laterad into short points, the space between filled with pale membrane. Basistyle, *b*, very long and slender, without lobes. Dististyle, *d*, about two-fifths as long as the basistyle, appearing as a simple slender terminal lobe, slightly curved, narrowed to the obtuse tip. Phallosome with the gonapophyses, *g*, unequally bispinous, the axial spine longer and more powerful than the lateral one.

73 (329). *CLADURA (CLADURA) JAPONICA* (Alexander).

Crypteria japonica ALEXANDER, Ent. Soc. America Ann. 11 (1918) 448.

Crypteria japonica ALEXANDER, Entomol. Mag. (Kyoto) 3 (1919) 125.

The types were from an unspecified locality in Honshu, presumably Kyoto (Akio Nohira) Collector's No. 26.

This, the first member of the genus to be discovered in Japan, was based only on female specimens. In the light of the great accession of species now known from the islands I feel very uncertain about the strict identity of this fly. It is possible that more material, including males, from the presumed type locality may help in settling the identity or otherwise the species may best be considered as falling as a species dubium.

74 (255). *CLADURA (CLADURA) MACHIDELLA* Alexander.

Cladura (Cladura) machidella ALEXANDER, Philip. Jour. Sci. 55 (1934) 50-51, pl. 1, fig. 19 (venation).

Cladura (Cladura) machidella ALEXANDER, Philip. Jour. Sci. 83 (1954) 295-296, pl. 4, fig. 40 (♂ hypopygium).

The unique type female was from Mount Hiei, Kyoto, Yamashiro, collected October 30, 1933, by Machida. The species later was found in Shikoku.

General coloration of entire body black, dusted with gray; fore femora black, the extreme bases obscure yellow; posterior femora with the outer third blackened, tarsi black; wings yellow, patterned with brown on the crossveins and the distal two-thirds of vein Cu; supernumerary crossveins in cells R₃ and R₄, in approximate transverse alignment with R₂.

75 (330). *CLADURA (CLADURA) MEGACAUDA* Alexander.

Cladura megacauda ALEXANDER, Philip. Jour. Sci. 31 (1926) 370-371, pl. 1, fig. 7 (venation), pl. 2, figs. 4, 5 (♂ hypopygium).

The type, a male, was from Sapporo, Ishikari, Hokkaido, taken in late August, 1925, by Satoru Kuwayama.

ECHIGO: Kurokawa, October 15, 1953, October 22, 1954 (*Baba*); Baba No. 290.

KOTSUKE: Hatomati Toge, Oze, September 22, 1950 (*Hasegawa*); Hiramino-Tashiro, Oze, September 7, 1952 (*Fukuhara*).

RIKUCHIU: Morioka, October 6, 1934 (*Kato*).

SHINANO: Sigakôgen, altitude 1,600 meters, September 11, 1953 (*Ito*).

UZEN: Mount Chokai, altitude 600 meters, October 17, 1950 (*Shirahata*).

76 (331). *CLADURA (CLADURA) MONACANTHA* Alexander. Plate 5, fig. 38.

Cladura monacantha ALEXANDER, Ent. Soc. America Ann. 40 (1947) 363-364.

The types were Inaba (Tottori-ken), collected by Sawada.

Male hypopygium (Plate 3, fig. 38) with the posterior border of tergite, *t*, slightly produced into a transverse glabrous plate, the mesal part of which is farther extended into two subcircular disks from very slender bases. Basistyle, *b*, moderately stout, less than twice the length of the dististyle, *d*, the latter terminal in position, stout, with the obtuse tip more or less pendant. Gonapophysis, *g*, distinctive, appearing as a narrow yellow blade, the apex narrowly obtuse, the outer margin before tip with a strong erect horn; margin of blade on same side as the horn with strong teeth; apex of blade beyond the horn with a few scattered pale setoid points. Ædeagus broad basally, narrowed to the tip, a little shorter than the apophyses.

76a (331a). *CLADURA (CLADURA) MONACANTHA FIMBRIATA* Alexander.

Cladura monacantha fimbriata ALEXANDER, Ent. Soc. America Ann. 40 (1947) 364.

The type, a male, was from Gora, Hakone District, collected November 11, 1931, by Sawada.

This weakly defined race is distinguished only by minor differences in the male hypopygium, including the narrower gonapophyses, with the lateral horn large and conspicuous; the outer setoid extensions continue down the margin as teeth, some of which are produced into hairlike points (Plate 5, fig. 38, subfigure on right).

77 (332). *CLADURA (CLADURA) NIPPONENSIS* Alexander.

Plate 5, fig. 39.

Cladura nipponensis ALEXANDER, Insec. Inscit. Menst. 7 (1919) 136.

Cladura nipponensis ALEXANDER, Philip. Jour. Sci. 31 (1926) 370, pl. 2, fig. 7 (♂ hypopygium, apophysis).

The types were from Tokyo and vicinity, taken November 16 to December 9, 1919, by Takahashi.

ECHIGO: Kurokawa, November 7 to 9, 1954 (*Baba*); *Baba* No. 326.

Male hypopygium (Plate 5, fig. 39) generally like that of *hakonensis*, differing especially in the details of structure of the phallosome, particularly the gonapophyses. Ninth tergite with the posterior border produced medially, the central part emarginate, the lateral angles produced into dusky flattened blades, the margins microscopically serrulate, in cases strongly so. Gonapophyses, *g*, deeply bifid, the axial spine longer and stouter than the lateral one.

78 (333.). *CLADURA* (*CLADURA*) *TELEPHALLUS* Alexander. Plate 5, fig. 40.

Cladura (*Cladura*) *telephallus* ALEXANDER, Ent. Soc. America Ann. 48 (1955) 373-374.

The types were from Oze, Hatomati Toge, Kotsuke, taken September 22, 1950, by Hasegawa.

KOTSUKE: Mount Tanigawa, altitude 700 to 800 meters, October 24, 1955 (*Baba*); *Baba* No. SD 38. Associated in nature with *Cladura* (*Cladura*) *brevifila* sp. nov. and *C. (C.) tetraspila* Alexander.

Male hypopygium (Plate 5, fig. 40) with the tergite, *t*, narrowed outwardly into a depressed-flattened plate that divides at apex into two obtuse lobes that are separated by a very narrow median split, in cases, the apex only slightly emarginate, without a further median split; no lateral tergal arms or projections. Basistyle, *b*, with the outer lobe shorter than the dististyle, stout, with long conspicuous setæ on outer third; mesal lobe elongate, provided with abundant microscopic spiculose points and scattered strong setæ. Dististyle, *d*, narrowed on outer third or less, the apex recurved, obtuse and slightly expanded, with long setæ. Phallosome, *p*, with the gonapophysis, *g*, short-stemmed, expanded into a glabrous oval flattened blade; ædeagus unusually long and extended, directed caudad and ventrad and finally cephalad, ending in a long filament that is about one-third the enlarged basal part, with a further pair of microscopic filaments at its base.

79 (334). *GONOMYIA* (*IDIOCERA*) *ARETE* sp. nov. Plate 6, figs. 41, 47.

Allied to *pruinosa*; pedicel and first flagellar segment yellowed; wings yellowish gray, stigma small, light brown; Sc short, vein R_4 nearly one-half longer than R_{2+3+4} ; male hypopygium with three styles, the intermediate and inner ones forked to produce a total of five branches or spines.

Male.—Length, about 4.5 millimeters; wing, 4.5.

Rostrum brownish black; palpi black. Antennæ with scape black, heavily gray pruinose; pedicel and base of first flagellar segment yellow, remainder of flagellum black; segments long-oval, with verticils that much exceed the segments. Head light gray.

Pronotum gray. Mesonotum discolored, the præscutum apparently gray with darker grayish brown stripes; scutal lobes brown, scutellum brown; postnotum darker brownish gray. Pleura light yellow, especially clear behind; dorsopleural region more infuscated. Halteres with stem pale, knob weakly infuscated. Legs with the coxæ and trochanters pale yellow; remainder of legs very slightly darkened, the outer tarsal segments more infuscated. Wings (Plate 6, fig. 41) yellowish gray, the costal border light yellow; stigma small, light brown, lying basad of vein R_3 ; cord slightly seamed with brown, best-evidenced by a deepening in color of the veins; remaining veins pale, with dark trichia. Venation: Sc_1 ending a short distance beyond the origin of R_s ; distance on costa between R_{1+2} and R_3 about one-third the length of the latter vein; vein R_4 relatively long, nearly one-half longer than R_{2+3+4} ; m-cu about one and one-half times its length before the fork of M.

Abdominal tergites brown, sternites paler; hypopygium brownish yellow. Male hypopygium (Plate 6, fig. 47) with the outer apical lobe of basistyle, *b*, exceeding one-half the length of the style, stout. Three dististyles, *d*, of which two are branched, producing a total of five styles or branches, these shaped as shown in figure; outer style simple, at extreme tip produced into two points arranged in virtually a straight angle; intermediate style largest, forked, the outer arm longest, appearing as a flattened blade that is extended into a long point, inner arm shorter, dilated on outer half, its outer apical angle produced into a darkened lobe; inner style forked, including a slender pale rod that narrows abruptly into an acute spine, and an inner very slender basal spine. Ædeagus constricted before the slightly dilated apex, with several strong setæ before the more narrowed yellow tip.

Habitat.—Japan (Honshu).

Holotype, male, Kurokawa, Echigo, August 23, 1955 (*Baba*); *Baba* No. 375.

The present fly is readily told from *Gonomyia* (*Idiocera*) *pruinosa* Alexander and *G. (I.) subpruinosa* Alexander, the

closest relatives, by the structure of the male hypopygium, the conformation of the dististyles being quite distinct.

80 (335.) **GONOMYIA (IDIOCERA) SUBPRUINOSA** Alexander. Plate 6, fig. 48.

Gonomyia (Ptilostena) subpruinosa ALEXANDER, Philip. Jour. Sci. 24 (1924) 588-590.

The types were from Jozankei, Hokkaido, taken on August 19, 1922 by Esaki. The species later was found at Koiwai Farm, near Morioka, Rikuchiu, from August 15 to September 2, 1920 by Teranishi.

Male hypopygium (Plate 6, fig. 48) with the outer lobe of the basistyle, *b*, large and fleshy, obtuse at tip. Five dististyles or branches of the same, *d*; outer style a slender blackened rod, long and sinuous, narrowed very gradually to the acute tip, on margin beyond midlength with a short spine; intermediate style deeply forked, the outer arm the longest element, appearing as a broadly flattened blade that is extended into a long slender spine, inner arm shorter, the blackened head bispinous; inner style forked at base, the inner arm a slender pale rod, its tip not produced into a spine, outer arm a long straight spine. Ædeagus slender, at tip narrowed into a point or spine.

The Formosan *Gonomyia (Idiocera) pruinosa* Alexander differs in the details of structure of the hypopygium, including the dististyles and ædeagus; outer dististyle a long pale very slender rod that narrows to an acute point, without a lateral spine, as in *subpruinosa*.

81 (336.) **GONOMYIA (IDIOCERA) TERANISHII** Alexander. Plate 6, fig. 49.

Gonomyia (Ptilostena) teranishii ALEXANDER, Ent. Soc. America Ann. 14 (1921) 118-119.

The type was from Koiwai Farm, near Morioka, Rikuchiu, taken August 17, 1920, by Teranishi.

ECHIGO: Kurokawa, July 3, 1954 (*Baba*); *Baba* No. 163. July 5, 1954 (*Baba*), No. 226; May 22, 1955 (*Baba*), No. 401.

What appears to represent the same species has been found as far to the west as Szechwan, China.

Male hypopygium (Plate 6, fig. 49) with the outer lobe of the basistyle, *b*, terminating in an acute blackened spine; inner apical lobe small and slender. Three dististyles, *d*, the outer one simple, narrowed gradually to an acute blackened point; intermediate style longest, stem long, forking into two unequal lobes, the outer one about twice as long as the inner and more slender, inner lobe blackened, its tip truncate; inner style nearly

as long as the outer one, pale, slender, on inner face with a low swelling that bears a concentration of setæ. *Ædeagus* blackened, straight, bifid at tip; lower margin with a row of about six long setæ.

82 (337). *LIPSOTHRIX BABAI* sp. nov.

Plate 6, figs. 44, 50.

General coloration of thorax black; antennæ of male relatively long; femora brownish yellow, tips narrowly blackened; wings weakly tinged with brown, with numerous macrotrichia in the outer cells; Rs very long and straight, exceeding vein R_4 ; m-cu about opposite midlength of the rectangular cell 1st M_2 ; male hypopygium with the interbase a long sinuous rod, near its tip bent at nearly a right angle into a slender spine.

Male.—Length, about 8 to 8.5 millimeters; wing, 8.5 to 9; antenna, about 2.5 to 2.9.

Female.—Length, about 10 millimeters; wing, 8.5; antenna, about 2.

Rostrum brown, small; palpi black. Antennæ with the scape and pedicel black, flagellum brown, elongate in male; flagellar segments oval, with a dense white pubescence and short verticils. Head dark brown; anterior vertex relatively narrow, elevated into a tubercle behind the antennal bases.

Pronotum brownish black, scutellum testaceous. Mesonotal præscutum and scutum polished black, scutellum and postnotum less distinctly polished black; parascutella, posterior callosities of the scutal lobes and the postnotal suture obscure yellow. Pleura black; dorsopleural region, meron and metapleura somewhat paler. Halteres with stem dirty white, clearer basally, knob weakly infuscated. Legs with the coxæ and trochanters testaceous yellow; femora obscure yellow to brownish yellow, the tips narrowly blackened; tibiæ light brown, the extreme base and tip slightly more darkened; tarsi brownish yellow, the outer two segments darker; claws with the teeth chiefly grouped at base. Wings (Plate 6, fig. 44) weakly tinged with brown, prearcular field more yellowed, costal field brownish yellow; veins brown. Strong macrotrichia in cells beyond cord (indicated in figure by stippling), chiefly in centers and outer ends of cells. Venation: Both Sc_1 and Sc_2 ending opposite or shortly before fork of Rs; R_{1+2} and R_2 subequal or the former a little longer; Rs very long and straight, longer than vein R_4 ; cell 1st M_2 rectangular, in cases longer and narrower, shorter than any of the veins beyond it; m-cu close to midlength of the cell.

Abdomen black, the centers of the more proximal sternites paler; hypopygium black. Male hypopygium (Plate 6, fig. 50) with the outer dististyle, *d*, beyond the lateral spine short and stout, narrowed to a terminal spine; inner style dilated on basal half, provided with a few long setæ. Interbase a long sinuous rod, near tip bent at nearly a right angle and narrowed into a slender acute spine. Ædeagus broad-based, behind its apex with two pale very obtuse flattened plates (not shown in figure).

Habitat.—Japan (Honshu).

Holotype, male, Kurokawa, Echigo, July 17, 1955 (*Baba*); Baba No. 448. Allotype, female, Mount Amakazari, Echigo, altitude 1,000 meters, June 25, 1955 (*Baba*); Baba No. AM 89. Paratopotype, male, July 13, 1955 (*Baba*); Baba No. 414. Paratypes, 2 males, one pinned with the allotype.

This very distinct and interesting crane-fly is another of the many discoveries of Dr. Kintaro Baba, to whom the species is dedicated. It is very distinct from all other Japanese members of the genus in the presence of strong macrotrichia in the outer wing cells. In this regard the fly agrees with western Nearctic species, such as *Lipsothrix nigrilinea* (Doane) and *L. shasta* Alexander, that differ in the coloration of the body, venation, and structure of the male hypopygium.

83 (338). *LIPSOTHRIX TOKUNAGAI* Alexander.

Lipsothrix tokunagai ALEXANDER, Philip. Jour. Sci. 50 (1933) 152, pl. 1, fig. 18 (venation), pl. 3, fig. 44 (♂ hypopygium).

The type was taken on Mount Daisen, Hoki, on July 2, 1931, by Masaaki Tokunaga, for whom it was named.

ECHIGO: Kurokawa, July 2 to 12, 1955 (*Baba*); Baba No. 444.

The present specimens are somewhat larger than the type (male, length, about 7.5 to 8 millimeters; wing, 7.5 to 8; antenna, about 2.2 to 2.3). The type male had lost the antennæ and legs and these are described herewith. Antennæ of male elongate, if bent backward extending about to midlength of the second abdominal segment, yellow throughout; flagellar segments with a dense white pubescence and short inconspicuous verticils. Legs uniformly whitened, with no indications of dark femoral tips, as in *apicifusca*. Male hypopygium as in the type. It may be noted that the interbase, as there figured, is slightly erroneous, being longer and more slender, strongly bent at near two-thirds the length, terminating in a long acute spine.

The very remarkable larvæ and pupæ of species of *Lipsothrix* have been discovered in recent years and have been discussed in two outstanding papers by Hinton and by Rogers and Byers.⁵

84 (339). RHABDOMASTIC (SACANDAGA) MICROXANTHA sp. nov. Plate 6, figs. 45, 51.

Size very small; wing of male 4 millimeters; color of entire body pale yellow; antennal flagellum brown; wings relatively narrow, pale yellow, veins very pale brownish yellow; relatively complete series of trichia on vein R_4 and distal sections of R_5 , M_{1+2} , M_3 and M_4 ; vein R_3 short and erect, about two-fifths the distance on C between veins R^{1+2} and R_3 ; cell 1st M_2 relatively long and narrow; male hypopygium with the spines of the outer dististyle relatively few but large and coarse; gonapophyses pointed at tips.

Male.—Length, about 3.5 millimeters; wing, 4.

Rostrum and palpi light yellow. Antennæ with scape and pedicel light yellow, flagellum brown; segments oval, much shorter than the longest verticils. Head light yellow.

Thorax uniformly very pale yellow. Halteres pale yellow. Legs yellow throughout. Wings (Plate 6, fig. 45) relatively narrow, pale yellow; veins very pale brownish yellow. Relatively complete series of long trichia on veins R_4 , distal sections of R_5 , M_{1+2} , M_3 and M_4 ; on R_1 basad almost to origin of R_s . Venation: Sc_1 ending nearly opposite midlength of the long R_s ; vein R_3 short and erect, about two-fifths as long as the distance on C between tips of R^{1+2} and R_3 ; cell 1st M_2 broad-based, relatively long and narrow, the length about three times the greatest width; m-cu shortly before midlength of M_{3+4} ; cell 2nd A relatively narrow.

Abdomen yellow throughout. Male hypopygium (Plate 6, fig. 51) with the outer dististyle, *d*, relatively slender, the spines few but large; inner style broad-based, narrowed rapidly to the slender apex. Gonapophysis, *g*, broad at base and apex, stem slender, tip pointed.

Habitat.—Japan (Honshu).

Holotype, male, Kurokawa, June 15, 1955 (*Baba*); *Baba* No. 398.

⁵Hinton, H. E. The structure of the spiracular gill of the genus *Lipsothrix* (Tipulidæ), with some observations on the living epithelium isolated in the gill at the pupa-adult moult. Roy. Ent. Soc. London Proc. (A) 30, pts. 1-3 (1955) 1-14, 14 figs.

Rogers, J. Speed, and George W. Byers. The ecological distribution, life-history, and immature stages of *Lipsothrix sylvia* (Diptera: Tipulidæ). Mus. Zool. Univ. Michigan Occas. Pap. 572 (1956) 1-14, 7 figs.

The only approximately similar regional species is the much larger *Rhabdomastix* (*Sacandaga*) *japonica* Alexander, which differs not only in size but in the details of coloration and venation, as the long Sc and the approximation of veins R_{1+2} and R_3 at the margin.

85 (340). RHABDOMASTIC (SACANDAGA) SADCENSIS sp. nov. Plate 6, fig. 46.

General coloration of thorax gray, the disk of the præscutum more infuscated but not forming an evident stripe; antennæ brownish black; halteres yellow; legs yellow throughout; wings tinged with gray, the prearcular and costal fields more yellowed; sparse macrotrichia on outer ends of veins R_5 , M_{1+2} and M_3 ; vein R_3 short, erect, the distance on C between R_{1+2} and R_3 fully twice the length of the latter.

Female.—Length, about 4 millimeters; wing, 4.3.

Rostrum gray; palpi black. Antennæ with scape and pedicel brownish black, flagellum broken. Head dark gray; anterior vertex broad, exceeding four times the diameter of the scape.

Prothorax gray. Mesonotum gray, the præscutum with the central area a trifle more infuscated but not forming distinct stripes; posterior sclerites of notum and the pleura somewhat lighter gray. Halteres yellow. Legs with the coxæ brownish testaceous, the fore pair somewhat darker; trochanters yellow; remainder of legs brownish yellow to yellowish brown throughout. Wings (Plate 6, fig. 46) tinged with gray, the prearcular and costal fields somewhat more yellowed; stigma not or scarcely indicated; veins pale brown, more brownish yellow in the brightened fields. Macrotrichia present on less than the distal half of outer section of vein M_3 , none on anterior branch of Rs. Venation: Sc_1 ending about opposite midlength of Rs, the latter about one-fifth longer than R_{2+3+4} ; vein R_3 short and erect, less than one-fourth R_4 , which is gently arcuated and curved slightly cephalad at tip; distance on C between R_{1+2} and R_3 fully twice the length of the latter vein; distal section of vein M_{1+2} arched at origin; m-cu at near midlength of M_{3+4} .

Abdominal tergites dark brown, sternites a little paler.

Habitat.—Japan (Honshu).

Holotype, female, Mount Donden, Sado, altitude 600 meters, July 19, 1955 (*Baba*); *Baba* No. SA 35.

The most similar regional species is *Rhabdomastix* (*Sacandaga*) *atrata* Alexander, which is brownish black throughout, including the halteres and legs.

ORMOSIA Rondani

Ormosia RONDANI, Prodr. Dipter. Ital. 1 (1856) 180.

Subgenus OREOPHILA Lachschewitz

Oreophila LACKSCHEWITZ, Troms. Mus. Arsh. 4 53 (1935) 7.

Oreophila LACKSCHEWITZ, Naturhist. Mus. Wien Ann. 50 (1939) 34-35 (published March 13, 1940); type *bergrothi* Strobl, 1894.

Ilisomyia RONDANI, Prodr. Dipter. Ital. 1 (1856) 180; type *nebulosa* Rondani (as *nubipennis* lapsus, nomen nudum).

Ilisomyia OSTEN SACKEN, Berlin. Ent. Zeitschr. 31 (1887) 231.

I consider the identity of *Ilisomyia* Rondani as being too much in doubt to be accepted in favor of the later proposed *Oreophila* Lachschewitz. The following species in northeastern Asia belong here—*Ormosia* (*Oreophila*) *confluente* Alexander (Japan), *O. (O.) weymarni* Alexander (Korea, Manchuria), and *O. (O.) yankovskyi* Alexander (Korea). Whether *Ormosia tokionis* Alexander should likewise be placed here is more questionable and its assignment to the present group would require a modification of characters in both the venation and the male hypopygium. Various other species, including *O. (O.) absaroka* Alexander and *O. (O.) flaveola* (Coquillett), occur in western and northwestern North America.

Male hypopygium with the phallosomic plate large, depressed-flattened, somewhat as in *Molophilus*; ædeagus much more slender than in typical *Ormosia*; usually with two dististyles. Wings with cell M_2 open by the atrophy of m.

86 (341). ORMOSIA (ORMOSIA) AMAKAZARII sp. nov. Plate 7, figs. 54, 56.

Allied to *nantaisana*; general coloration dark gray, the præscutum with a vaguely indicated darker central stripe; pseudo-sutural foveæ black; halteres light yellow; legs brownish black, femoral bases obscure yellow, including about the basal third of the fore femora; wings grayish yellow, the prearcular and costal fields clear light yellow, stigma dark brown; cell 1st M_2 closed; male hypopygium with the tergite produced medially; dististyles relatively small, the outer style with rows of microscopic spicules on the weakly capitate outer end; phallosome simple, gonapophyses comparatively small, ædeagus very broad.

Male.—Length, about 5.5 millimeters; wing, 6.5; antenna, about 1.3.

Rostrum dark gray; palpi black. Antennæ black throughout; flagellar segments passing through oval to elongate-oval, the

verticils of moderate length, longer and stouter on the proximal segments. Head gray; anterior vertex broad.

Pronotum dark plumbeous gray, the sides of the scutellum obscure yellow. Mesonotum dark gray, the præscutum with a vaguely indicated darker central stripe; pseudosutural foveæ and tuberculate pits black; pronotum with very long erect setæ. Pleura and pleurotergite dark gray, including the dorsopleural membrane. Halteres light yellow. Legs with the coxæ dark gray; trochanters obscure yellow; remainder of legs brownish black, the femoral bases obscure yellow, on the fore legs including about the basal third, on the posterior legs more than one-half. Wings (Plate 7, fig. 54) with the ground grayish yellow, the prearcular and costal fields clear light yellow; stigma dark brown, conspicuous; outer end of cell R_3 and a seam in cell M adjoining vein Cu vaguely darker; veins brown, yellow in the flavous parts. Macrotrichia distributed virtually over the entire wing surface (position shown in figure by stippling). Venation: Sc_1 ending just beyond level of R_2 , the latter at fork of R_{2+3+4} ; m-cu about one-fourth its length beyond the fork of M.

Abdomen, including hypopygium, dark brown, with abundant long yellow setæ. Male hypopygium (Plate 7, fig. 56) with the ninth tergite, *t*, very short and broad, the median area of the posterior border produced into a subquadrate lobe. Both dististyles, *d*, relatively small, as compared with *nantaisana* and *subalpina*; outer style with rows of microscopic spinules on outer or head portion; inner style cleaverlike, with microscopic setæ near apex. Phallosome, *p*, simple, as compared with related forms; gonapophyses appearing as comparatively small flattened blades, dark-colored with pale margins. Ædeagus very broad at base, narrowed outwardly, the tip truncate.

Habitat.—Japan (Honshu).

Holotype, male, Mount Amakazari, Echigo, altitude 1,000 meters, June 25, 1955 (*Baba*); Baba No. AM 35.

The most similar described species are *Ormosia* (*Ormosia*), *nantaisana* Alexander and *O. (O.) subalpina* Alexander, which are most readily distinguished by the structure of the male genitalia, as indicated above. Both of these species are further discussed later in this report (Nos. 87, 88).

87 (206). *ORMOSIA (ORMOSIA) NANTAISANA* Alexander.

Plate 7, fig. 57.

Ormosia nantaisana ALEXANDER, Philip. Jour. Sci. 82 (1953) 304 (latest reference).

SHIMOTSUKE: Nantaisan, August 16, 1909 (type).

SHINANO: Mount Shirouma, August 8, 1931 (*Machida* and *Nakamura*). Kamikochi, August 18 to 21, 1934 (*Kariya*).

Male hypopygium (Plate 7, fig. 57) with the tergite, *t*, transverse, the central part with an X-shaped thickening, the anterior arms extended outwardly into blunt lobes, the median area with pale membrane. Basistyle, *b*, with the interbasal process (or lateral gonapophysis) appearing as an inner slender pale blade and a much larger outer or lateral expansion. Outer dististyle, *d*, short and stout, its apex expanded and more or less folded, provided with dense rows of spiculate points; inner style a longer cultrate yellow blade. Phallosome, *p*, including conspicuous yellowish horn-colored gonapophyses and a smaller ædeagus, the latter somewhat as in the subgenus *Rhypholophus* but with the arms short and inconspicuous.

88. (342). *ORMOSIA (ORMOSIA) SUBALPINA* Alexander.

Plate 7, fig. 58.

Ormosia (Ormosia) subalpina ALEXANDER, Ent. Soc. America Ann. 40 (1947) 366-367.

The types and only known specimens were from Mount Kurobegoro, Etchu, altitude 2,400 meters, taken August 8, 1931, by K. Imanishi.

The present fly, while allied to *Ormosia (Ormosia) nantaisana*, is quite distinct in the structure of the male hypopygium.

Male hypopygium (Plate 7, fig. 58) with the tergite generally as in *nantaisana*, the posterior border produced and emarginate, the lateral lobes slender. Basistyle, *b*, short and stout. Dististyles, *d*, unusually large, the outer style very short-stemmed, the head large, the total length only about one-half greater than the breadth near apex, the latter with rows of dense black spicules; inner style unusually large, its length virtually equal to that of the basistyle or about one-half longer than the outer style, roughly cultriform in outline. Phallosome, *p*, with the lateral apophyses appearing as very large incurved pale blades, expanded at outer ends; inner apophyses much smaller; ædeagus with two genital ducts, each unequally bifid at tip, the inner part produced into a long slender point.

The three species last considered, *Ormosia amakazarii*, *O. nantaisana*, and *O. subalpina*, are aberrant in the typical subgenus and show certain points of resemblance to the subgenus

Rhypholophus Kolenati, to which group they might more properly be referred. More material or the discovery of annectant forms might help clarify this question.

ERIOPTERA Meigen

Erioptera MEIGAN, Illiger's Magaz. 2 (1803) 262.

Subgenus TASIOCERODES novum

Characters as in the typical subgenus, differing especially in the venation, particularly the radial field which is as much as the otherwise very distinct genus *Tasiocera*; vein R_{2+3} ; perpendicular at the end of R_s , bent at a right angle, at the bend with R_2 directed basad, thence angularly bent to joint R_1 ; R_{4+5} lacking, with both R_4 and R_5 at the end of R_s , the former in direct longitudinal alignment with it; basal section of R_5 in direct transverse alignment with R_{2+3} ; vein 2nd A. sinuous. In a second species, *subsessilis*, there is a very short element R_{2+3+4} at the end of R_s , this soon forking into R_4 . Veins with very long trichia, approximately as in *Molophilus*. Male hypopygium of the general type of *Erioptera*; two complex dististyles; phallosome likewise complex, with long apophyses.

Type of subgenus.—*Erioptera* (*Tasiocerodes*) *persessilis* sp. nov. (Japan). Also included is *E. (T.) subsessilis* Alexander, of Formosa.⁶ Since there are only the two species known at present, and in order to complete the data I am providing a figure and brief description of the male hypopygium of *subsessilis*. This agrees with the present fly in its general structure but all details are distinct (Plate 8, fig. 62). Dististyles, *d*, complex, the larger outer one with the beaklike portion long and slender; inner style shaped about as figured. Phallosome, *p*, with the central plate or ædeagus broad-based, with spinulose outer margins, the outer spines large and blackened; lateral apophyses appearing as slender ribbonlike blades that are narrowed only at the slightly darkened tips (only one side of phallosome is figured). The various parts as described and figured are as they appear on a microscope slide and may appear different at various other views or angles.

89 (343). ERIOPTERA (TASIOCERODES) PERSESSILIS sp. nov. Plate 7, fig. 53;
Plate 8, fig. 61.

General coloration of mesonotum brownish yellow, vaguely patterned with darker; antennæ testaceous yellow; knob of halteres infuscated; legs yellow; wings subhyaline, venation as described under the subgenus; male hypopygium with both

⁶ Ent. Soc. America Ann. 17 (1924) 67-68.

dististyles complex, lateral apophyses appearing as yellow rods that narrow into long straight blackened spines.

Male.—Length, about 3.4 to 3.5 millimeters; wing, 3.8 to 4; antenna, about 1.0.

Rostrum brownish yellow; palpi brownish black. Antennæ testaceous yellow, the outer segments darker; flagellar segments long-oval to fusiform, with coarse bristles that are much longer than the segments. Head above chiefly yellow, with coarse black bristles.

Cervical region darkened; pronotum obscure yellow. Mesonotum brownish yellow, with vague indications of a darker pattern on the præscutum, especially evident on the interspaces where there are coarse black setæ; posterior sclerites of notum obscure yellow. Pleura and pleurotergite paler yellow. Halteres with stem yellow, knob infuscated. Legs with the coxæ and trochanters pale yellow; remainder of legs yellow, the outer tarsal segments a trifle darker. Wings (Plate 7, fig. 53) subhyaline, the veins and trichia a little darker. Venation: As in the subgenus; vein 2nd A sinuous.

Abdominal tergites brownish yellow, sternites clearer yellow; hypopygium light brown. Male hypopygium (Plate 8, fig. 61) generally as in *subsessilis* (Plate 8, fig. 62) but differing in all details. Basistyle, *b*, with the inner apical angle produced into a long blade that is provided with long setæ. Two dististyles, *d*, both complex in structure, the outer a narrow blade with a greatly expanded basal flange; inner style arising very low on the basistyle, narrowed and sinuous at base, expanded outwardly, terminating in a spinous point. Phallosome, *p*, complex, including a pair of broad flattened plates that apparently represent the ædeagus, these terminating in blackened divergent spines, at base of plates near the midline with a slender acute spine; lateral margins of plates with microscopic retrorse spinules; lateral apophyses much longer, appearing as yellow rods that narrow gradually into long straight black spines.

Habitat.—Japan (Honshu).

Holotype, male, Kurokawa, Echigo, July 30, 1955 (*Baba*); *Baba* No. 379. Paratopotype, male, July 9, 1955; *Baba* No. 359.

The distinctions between this species and the Formosan *Erioptera* (*Tasiocerodes*) *subsessilis* Alexander are indicated in the description.

90 (344). ERIOPTERA (HOPLERIOPTERA) HONSHUENSIS sp. nov. Plate 7, fig. 52;
Plate 8, fig. 60.

General coloration of body dull yellow, without pattern; femora brownish black, their bases restrictedly paler, narrowest on the fore legs; wings hyaline, unpatterned; m-cu at near two-thirds the length of M_{3+4} ; male hypopygium with the outer dististyle a simple paddlelike blade, the outer half densely setuliferous; inner style shorter, the outer margin with an erect slender spine; phallosome with the gonapophyses appearing as simple black horns.

Male.—Length, about 4 to 4.5 millimeters; wing, 5 to 5.3.

Female.—Length, about 5 to 5.5 millimeters; wing, 5 to 5.5

Rostrum yellow; palpi brown. Antennæ with the scape brownish yellow, pedicel brown, flagellum light brown; flagellar segments elongate, with conspicuous verticils. Head yellow.

Thorax dull yellow, without evident pattern. Halteres pale yellow. Legs with the coxæ and trochanters pale yellow; femora brownish black, the bases restrictedly paler, narrowest on the fore legs, more extensive on the middle and posterior pairs; remainder of legs dark brown to brownish black. Wings (Plate 7, fig. 52) hyaline, unpatterned; veins pale. Venation: Sc_1 ending about opposite the fork of R_{2+3+4} , Sc_2 shortly before fork of Rs; R_2 just beyond fork of R_{2+3+4} , vein R_{2+3} short to lacking; cell 1st M_2 closed; m-cu far distad, at near two-thirds M_{3+4} ; vein 2nd A nearly straight.

Abdomen, including hypopygium, obscure yellow. Male hypopygium (Plate 8, fig. 60) with the tergite poorly delimited. Basistyle, *b*, simple, the mesal-apical angle produced into a small lobule. Outer dististyle, *d*, a long blackened blade, the outer two-thirds more expanded, provided with abundant microscopic setulæ; inner style about two-thirds as long, appearing as a paler blade, on outer margin beyond midlength with an erect to slightly recurved slender black spine that has about three long setæ around its base; a low tubercle beyond the spine on face of blade bears three similar very long setæ. Phallosome, *p*, unusually simple; gonapophyses appearing as slender gently curved smooth black horns, their bases enlarged; ædeagus longer, gradually narrowed to the simple apex.

Habitat.—Japan (Honshu).

Holotype, male, Mount Amakazari, Echigo, altitude 900 meters, June 25, 1955 (*Baba*); Baba No. AM 42. Allotopotype, female. Paratopotypes, 6 males and females.

Erioptera (*Hoploerioptera*) *honshuensis* is quite distinct in hypopygial structure from the two species of the subgenus previously described, *E. (H.) luctuosipes* Alexander and *E. (H.) shikokuensis* Alexander, both from Shikoku.

91 (345). **ERIOPTERA (PSILOCONOPA) MACHIDAI** Alexander. Plate 7, fig. 55.

Erioptera (Ilisia) machidai ALEXANDER, Philip. Jour. Sci. 44 (1931) 363-364, pl. 1, fig. 19 (venation).

The type, a female, was from Hirayu, Shinano, taken July 27, 1929, by Machida.

ECHIGO: Mount Amakazari, altitude 900 meters, June 25, 1955 (*Baba*); *Baba* No. AM 41.

SHINANO: Kamikochi, altitude 5,000 feet, August 18 to 21, 1934 (*Kariya*).

Male hypopygium (Plate 7, fig. 55) with the tergite large, its posterior border convexly rounded, with strong setæ. Basistyle, *b*, stout, narrowed to an obtuse apical point. Outer dististyle, *d*, very large and complex, blackened, shaped about as figured; outer margin with two small acute points, with a further large black spine lying across the face of the style, directed outwardly; apex of rostral portion broad, truncated; inner style small, pale, gradually narrowed outwardly. Phallosome, *p*, very complex, on either side of the broad ædeagus with three apophyses, the innermost appearing as a slender straight black rod, at apex with about six teeth; lateral of this a longer and stouter apophysis, outer half blackened, outer end with more numerous small teeth; at base of these rods with a large pale subtending blade, virtually as long as the longest apophysis.

92 (346). **ERIOPTERA (ERIOPTERA) DAMA** sp. nov.

Plate 8, figs. 59, 63.

Size relatively large (wing of male over 6 millimeters); general coloration of entire body light yellow; antennæ yellow; halteres with stem whitened, knob a trifle darker; legs yellow; wings pale yellow, the outer radial field, including the stigma, more saturated yellow; male hypopygium with the outer dististyle very complex, with four branches that bear smaller denticles; inner style a long slender simple rod; gonapophysis a blackened rod, its outer margin with microscopic denticles.

Male.—Length, about 5.5 millimeters; wing, 6.2.

Rostrum light yellow; palpi with first segment yellow, the remainder abruptly and conspicuously blackened. Antennæ

with scape and pedicel fulvous yellow, flagellum clear light yellow. Head light yellow; eyes large, black, contiguous beneath.

Thoracic dorsum pale yellow, the præscutum a trifle more fulvous yellow medially in front; pleura almost white. Halteres with the stem whitened, knob a trifle darker. Legs with the coxæ and trochanters pale yellowish white; remainder of legs yellow. Wings (Plate 8, fig. 59) pale yellow, the outer radial field, including the stigma, more saturated yellow; veins yellow, the trichia pale. Venation: Vein 2nd A strongly sinuous on outer third.

Abdomen, including hypopygium, yellow, with only certain structures of the genitalia black. Male hypopygium (Plate 8, fig. 63) with the basistyle, *b*, relatively simple, not produced into an apical lobe. Dististyles, *d*, very complex, especially the outer style which is much branched, suggesting the specific name. In the unique type slide, one style is lacking and the tips of certain of the branches broken so the appearance of the hypopygium may be somewhat different from the figure, as provided; outer style blackened, with the main branch broad-based, forking into two unequal arms, the longer one with five or six small denticles scattered along the inner margin, shorter arm smooth; near base of style with apparently two distinct arms, one of which is broken near its base; inner style a long slender simple rod, paler and more dilated at base, blackened outwardly, the tip broken. Phallosome with the gonapophyses, *g*, appearing as nearly straight blackened rods, the outer margins with microscopic denticles. Arms of ædeagus long and slender.

Habitat.—Japan (Honshu).

Holotype, male, Kurokawa, Echigo, June 17, 1955 (*Baba*); *Baba* No. 443.

A very distinct species, most similar to *Erioptera* (*Erioptera*) *holoxantha* Alexander, differing evidently in the complicated outer dististyle of the male hypopygium.

93 (347). *MOLOPHILUS* (*MOLOPHILUS*) *DIFEROX* sp. nov.

Plate 8, fig. 64.

Belongs to the *gracilis* group and subgroup; allied to *ferox*; general coloration black, pretergites yellow; wings weakly tinged with brown, veins and trichia darker; male hypopygium with the outer lobe of basistyle deeply and unequally bifid; both

dististyles simple, from dilated bases, the concave margins with microscopic spinules.

Male.—Length, about 3.5 millimeters; wing, 4.2; antenna, about 0.9.

Rostrum and palpi black. Antennæ black throughout, short; flagellar segments oval to long-oval, with very long verticils. Head dull black.

Pronotum black, pretergites light yellow. Mesonotum dull black, opaque by a sparse pruinosity; pleura black. Halteres yellow, knob large, somewhat more obscured. Legs with the coxæ brownish yellow; trochanters yellow; femora brown, yellowed basally, the tips passing into dark brown; tibiæ brownish yellow, the tips more narrowly and vaguely darkened; tarsi dark brown. Wings with a weak brownish tinge, the prearcular and costal fields slightly more yellowed; veins and macrotrichia brown. Venation: R_2 lying shortly beyond level of $r-m$; petiole of cell M_3 about three times $m-cu$; vein 2nd A relatively long, extending to shortly beyond the level of $m-cu$.

Abdomen, including hypopygium, brownish black. Male hypopygium (Plate 8, fig. 64) with the dorsal lobe of the basistyle, *b*, small, only about twice as long as broad, provided with a few setæ; lateral lobe very large and conspicuous, simulating the dististyles in length and shape, broad at base, deeply bifid into a slender outer arm and a shorter but broader inner arm that narrows into a slender curved spine; ventral lobe broad. Dististyles, *d*, two, both appearing as simple curved rods from dilated bases, the outer style more slender, narrowed very gradually to a long slender spine, the concave margin with abundant spinules, more sparse and scattered outwardly; inner style a trifle shorter but stouter, tip more obtuse, the face and margin with six or seven small scattered spinules. Phallosomic plate, *p*, oval, microscopically setuliferous. Ædeagus very long and slender.

Habitat.—Japan (Honshu).

Holotype, male, Kurokawa, Echigo, altitude 200 meters, June 12, 1955 (*Baba*); *Baba* No. 380.

The various regional species that are allied to *Molophilus* (*Molophilus*) *ferox* Alexander, including also *M. (M.) efferox* Alexander and *M. (M.) perferox* Alexander, all are separated among themselves by differences in the male hypopygium.

94 (348). **MOLOPHILUS (MOLOPHILUS) KIUSHIUENSIS** Alexander. Plate 8, fig. 65.

Molophilus (Molophilus) kiushiuensis ALEXANDER, Philip. Jour. Sci. 73 (1940) 416-417, pl. 1, fig. 24 (venation), pl. 5, fig. 50 (♂ hypopygium).

The types were from Fugandake, Kyushu, collected in May, 1922.

ECHIGO: Sugatani, Kitakanbara, altitude 300 meters, May 8, 1955 (*Hiroshi Koike*); Baba No. 466.

I now consider this fly as being a member of the *gracilis* group, *pubipennis* subgroup of the genus, rather than as belonging to the typical subgroup, as was originally indicated.

Male hypopygium (Plate 8, fig. 65) with the dorsal lobe of the basistyle, *b*, relatively slender; ventral lobe small, with spinulose points, additional to the scattered setæ. Outer dististyle, *d*, broad, especially at midlength, the tip obtuse and indistinctly bidentate; spicules on face of style about 15 to 18 in number; inner style with the apical point longer than in type (as shown in subfigure on extreme right), with a few microscopic denticles at point of bending. Phallosomic plate subtriangular, narrowed on outer part, microscopically setulose. Ædeagus small, only about one-third to one-half longer than the phallosome.

95 (349). **MOLOPHILUS (MOLOPHILUS) PERFLAVEOLUS** Alexander.

Molophilus perflaveolus ALEXANDER, Can. Ent. 50 (1918) 160-161.

Molophilus auricomus ALEXANDER, Insec. Inscit. Menst. 14 (1926) 115-117.

Molophilus auricomus ALEXANDER, Ins. Connecticut, 6 Dipt. (1942) 478, pl. 55 B (♂ hypopygium).

The species was described from North America, where it is known to have a very discontinuous distribution, in the east occurring in the southern Appalachians, and again throughout the far western states and provinces. The discovery of the fly in Japan is of unusual interest.

ECHIGO: Kurokawa, June 28, 1954 (*Baba*); Baba No. 199.

96 (350). **MOLOPHILUS (MOLOPHILUS) POLYCANTHUS** Alexander. Plate 8, fig. 66.

Molophilus polycanthus ALEXANDER, Philip. Jour. Sci. 59 (1936)

254-255, pl. 1, fig. 24 (venation), pl. 2 fig. 38 (♂ hypopygium).

The types were from Kibune, Kyoto, Yamashiro, taken August 17, 1932, by Tokunaga.

ECHIGO: Kurokawa, July 11, 1954 (*Baba*); Baba No. 202. August 1, 1955 (*Baba*); Baba No. 378.

The original description and figure of the male hypopygium were somewhat incomplete and incorrect in certain details.

The further material now available indicates the great complexity of parts in this organ.

Male hypopygium (Plate 8, fig. 66) with the basistyle, *b*, strikingly armed with spines. Dorsal lobe small, simple; ventral lobe long and fleshy, with strong setæ; remaining lobes of basistyle extended into spinelike points, including a mesal lobe that bears a needlelike spine from an expanded base, with a second similar spine from still nearer the base; a further small spinous point, as shown in the figure (drawn separately, to left of main figure). Dististyles, *d*, apparently two, the longer one a powerful black rod from an expanded base, its outer two-thirds more slender, before tip with several strong spinulose points; what appears to be the second style is a strong black spine from an expanded base, near origin produced into a short yellow arm. Phallosome, *p*, obtuse, glabrous. *Ædeagus* weakly expanded at base, gradually narrowed outwardly, approximately three times as long as the phallosome.

The unusual array of spinous points on the hypopygium serves to separate the present fly from similar forms. It is believed that the two structures discussed as being dististyles are such but with the material available there is some possibility that some of the spines of the basistyle and the dististyles may have been confused.

ILLUSTRATIONS

[Legend; *a*, sēdeagus; *b*, basistyle; *d*, dististyle; *g*, gonapophysis;
i, interbase; *p*, phallosome; *t*, tergite.]

PLATE 1

- FIG. 1. *Pedicia* (*Pedicia*) *brachycera* Alexander; male hypopygium.
 2. *Pedicia* (*Pedicia*) *cubitalis* Alexander; male hypopygium.
 3. *Pedicia* (*Pedicia*) *gaudens* (Alexander); male hypopygium.
 4. *Pedicia* (*Pedicia*) *subtransversa triacantha* subsp. nov.; male hypopygium.
 5. *Pedicia* (*Tricyphona*) *confluens subconfluens* subsp. nov.; venation.
 6. *Pedicia* (*Tricyphona*) *diaphanoides* Alexander; venation.

PLATE 2

- FIG. 7. *Pedicia* (*Tricyphona*) *confluens subconfluens* subsp. nov.; male hypopygium.
 8. *Pedicia* (*Tricyphona*) *kirishimensis* Alexander; male hypopygium.
 9. *Pedicia* (*Tricyphona*) *norikuræ* sp. nov.; male hypopygium.
 10. *Pedicia* (*Tricyphona*) *seticauda* (Alexander); male hypopygium.
 11. *Pedicia* (*Tricyphona*) *setipennis* (Alexander); male hypopygium.
 12. *Pedicia* (*Tricyphona*) *vetusta* (Alexander); male hypopygium.
 13. *Pedicia* (*Tricyphona*) *diaphanoides* Alexander; male hypopygium.

PLATE 3

- FIG. 14. *Nipponomyia pentacantha* sp. nov.; venation.
 15. *Heterangæus laticinctus* Alexander; venation.
 16. *Nipponomyia kuwanai* (Alexander); male hypopygium.
 17. *Nipponomyia pentacantha* sp. nov.; male hypopygium.
 18. *Nipponomyia trispinosa* (Alexander); male hypopygium.
 19. *Heterangæus laticinctus* Alexander; male hypopygium.

PLATE 4

- FIG. 20. *Dicranota* (*Rhaphidolabis*) *babai* sp. nov.; venation.
 21. *Dicranota* (*Rhaphidolabis*) *basistylata* sp. nov.; venation.
 22. *Dicranota* (*Rhaphidolabis*) *trilobulata* sp. nov.; venation.
 23. *Dicranota* (*Rhaphidolabis*) *babai* sp. nov.; male hypopygium.
 24. *Dicranota* (*Rhaphidolabis*) *basistylata* sp. nov.; male hypopygium.
 25. *Dicranota* (*Rhaphidolabis*) *macracantha* Alexander; male hypopygium.
 26. *Dicranota* (*Rhaphidolabis*) *ontakensis* Alexander; male hypopygium.
 27. *Dicranota* (*Rhaphidolabis*) *trilobulata* sp. nov.; male hypopygium.
 28. *Dicranota* (*Rhaphidolabis*) *profunda* Alexander; male hypopygium.
 29. *Dicranota* (*Dicranota*) *sicaria* Alexander; male hypopygium.

PLATE 5

- FIG. 30. *Cladura* (*Cladura*) *brevifila* sp. nov.; venation.
 31. *Cladura* (*Cladura*) *autumna* Alexander; male hypopygium.
 32. *Cladura* (*Cladura*) *babai* Alexander; male hypopygium.
 33. *Cladura* (*Cladura*) *bidens* Alexander; male hypopygium.
 34. *Cladura* (*Cladura*) *bicornuta* Alexander; male hypopygium.
 35. *Cladura* (*Cladura*) *brevifila* sp. nov.; male hypopygium.
 36. *Cladura* (*Cladura*) *daimio* Alexander; male hypopygium.
 37. *Cladura* (*Cladura*) *hakonensis* Alexander; male hypopygium.
 38. *Cladura* (*Cladura*) *monacantha* Alexander; male hypopygium.
 39. *Cladura* (*Cladura*) *nipponensis* Alexander; male hypopygium.
 40. *Cladura* (*Cladura*) *telephallus* Alexander; male hypopygium.

PLATE 6

- FIG. 41. *Gonomyia* (*Idiocera*) *arete* sp. nov.; venation.
 42. *Gonomyia* (*Idiocera*) *subpruinosa* Alexander; venation.
 43. *Gonomyia* (*Idiocera*) *teranishii* Alexander; venation.
 44. *Lipsothrix* *babai* sp. nov.; venation.
 45. *Rhabdomastix* (*Sacandaga*) *microxantha* sp. nov.; venation.
 46. *Rhabdomastix* (*Sacandaga*) *sadensis* sp. nov.; venation.
 47. *Gonomyia* (*Idiocera*) *arete* sp. nov.; male hypopygium.
 48. *Gonomyia* (*Idiocera*) *subpruinosa* Alexander; male hypopygium.
 49. *Gonomyia* (*Idiocera*) *teranishii* Alexander; male hypopygium.
 50. *Lipsothrix* *babai* sp. nov.; male hypopygium.
 51. *Rhabdomastix* (*Sacandaga*) *microxantha* sp. nov.; male hypopygium.

PLATE 7

- FIG. 52. *Erioptera* (*Hoplærioptera*) *honshuensis* sp. nov.; male venation.
 53. *Erioptera* (*Tasiocerodes*) *persessilis* sp. nov.; venation.
 54. *Ormosia* (*Ormosia*) *amakazarii* sp. nov.; venation.
 55. *Erioptera* (*Psiloconopa*) *machidai* Alexander; male hypopygium.
 56. *Ormosia* (*Ormosia*) *amakazarii* sp. nov.; male hypopygium.
 57. *Ormosia* (*Ormosia*) *nantaisana* Alexander; male hypopygium.
 58. *Ormosia* (*Ormosia*) *subalpina* Alexander; male hypopygium.

PLATE 8

- FIG. 59. *Erioptera* (*Erioptera*) *dama* sp. nov.; venation.
 60. *Erioptera* (*Hoplærioptera*) *honshuensis* sp. nov.; male hypopygium.
 61. *Erioptera* (*Tasiocerodes*) *persessilis* sp. nov.; male hypopygium.
 62. *Erioptera* (*Tasiocerodes*) *subsessilis* Alexander; male hypopygium.
 63. *Erioptera* (*Erioptera*) *dama* sp. nov.; male hypopygium.
 64. *Molophilus* (*Molophilus*) *diferox* sp. nov.; male hypopygium.
 65. *Molophilus* (*Molophilus*) *kiushiuensis* Alexander; male hypopygium.
 66. *Molophilus* (*Molophilus*) *polycanthus* Alexander; male hypopygium.



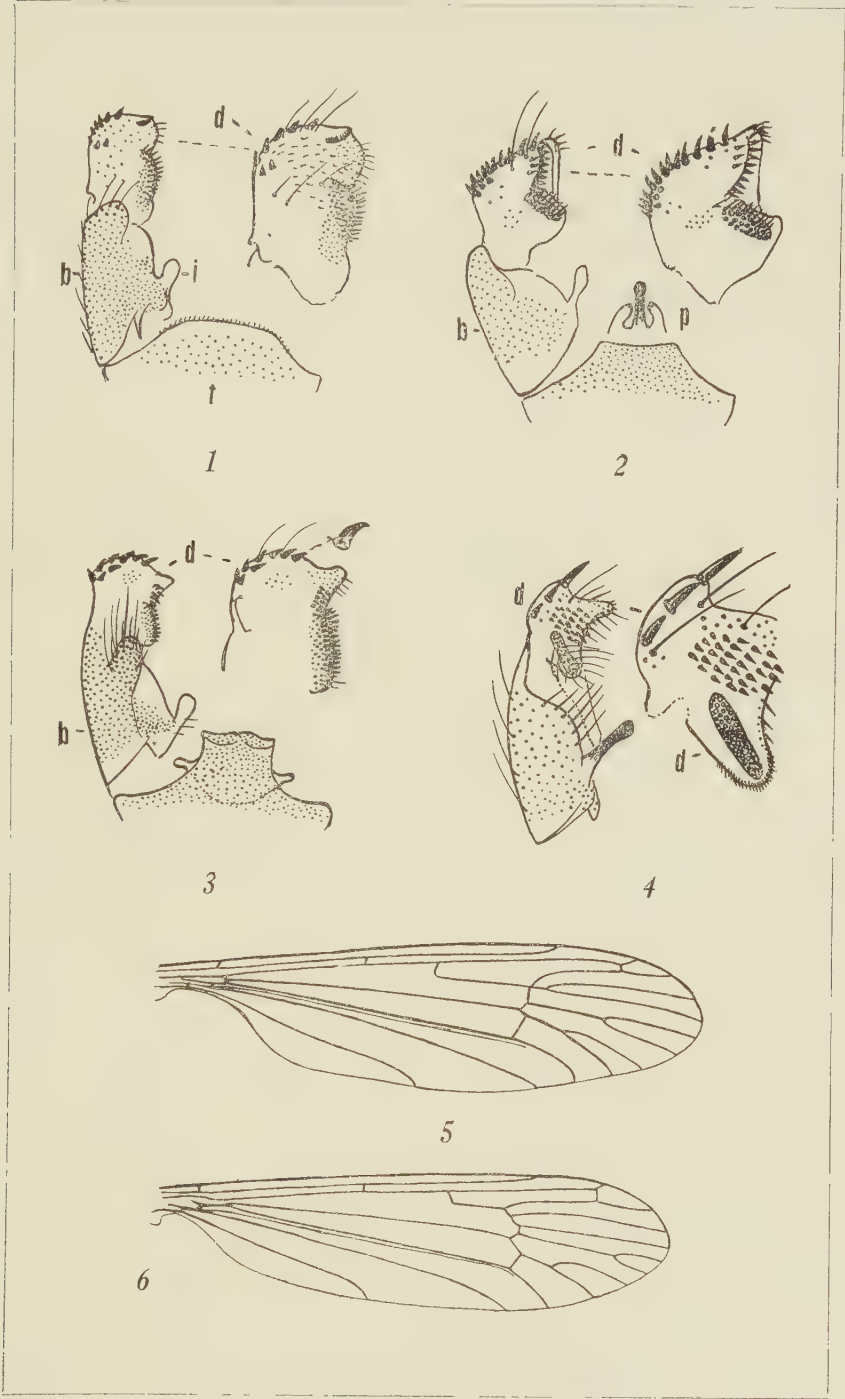


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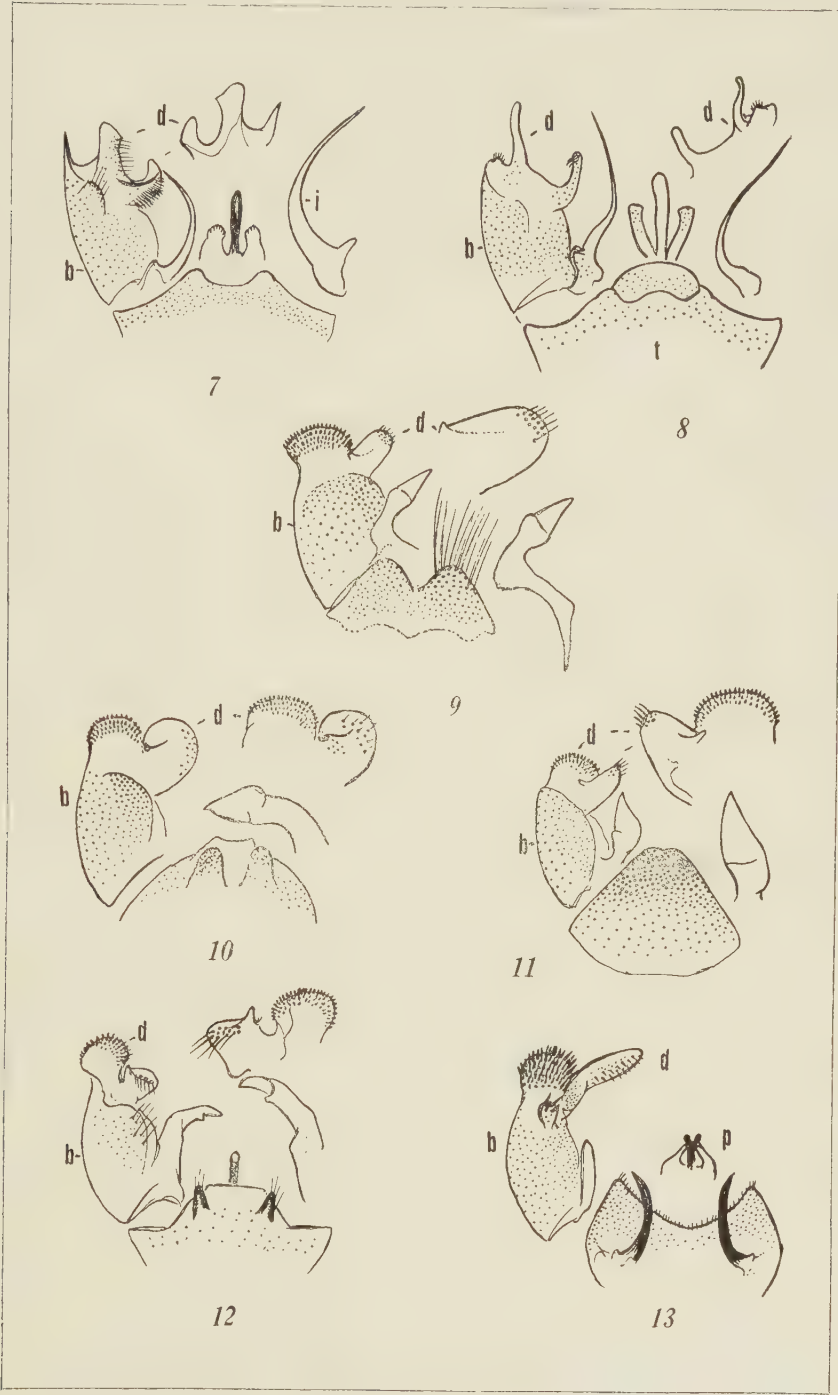
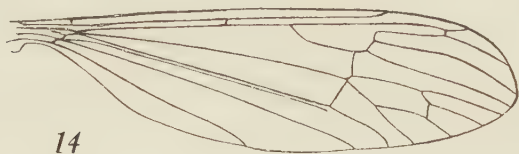
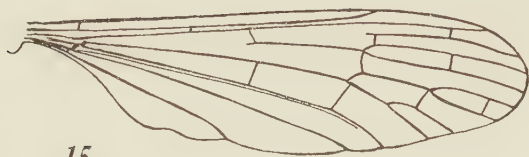


PLATE 2.



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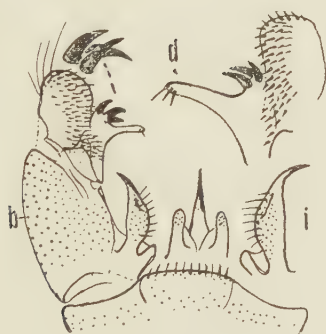
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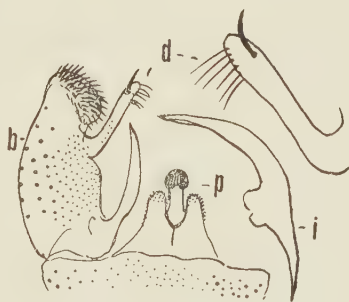
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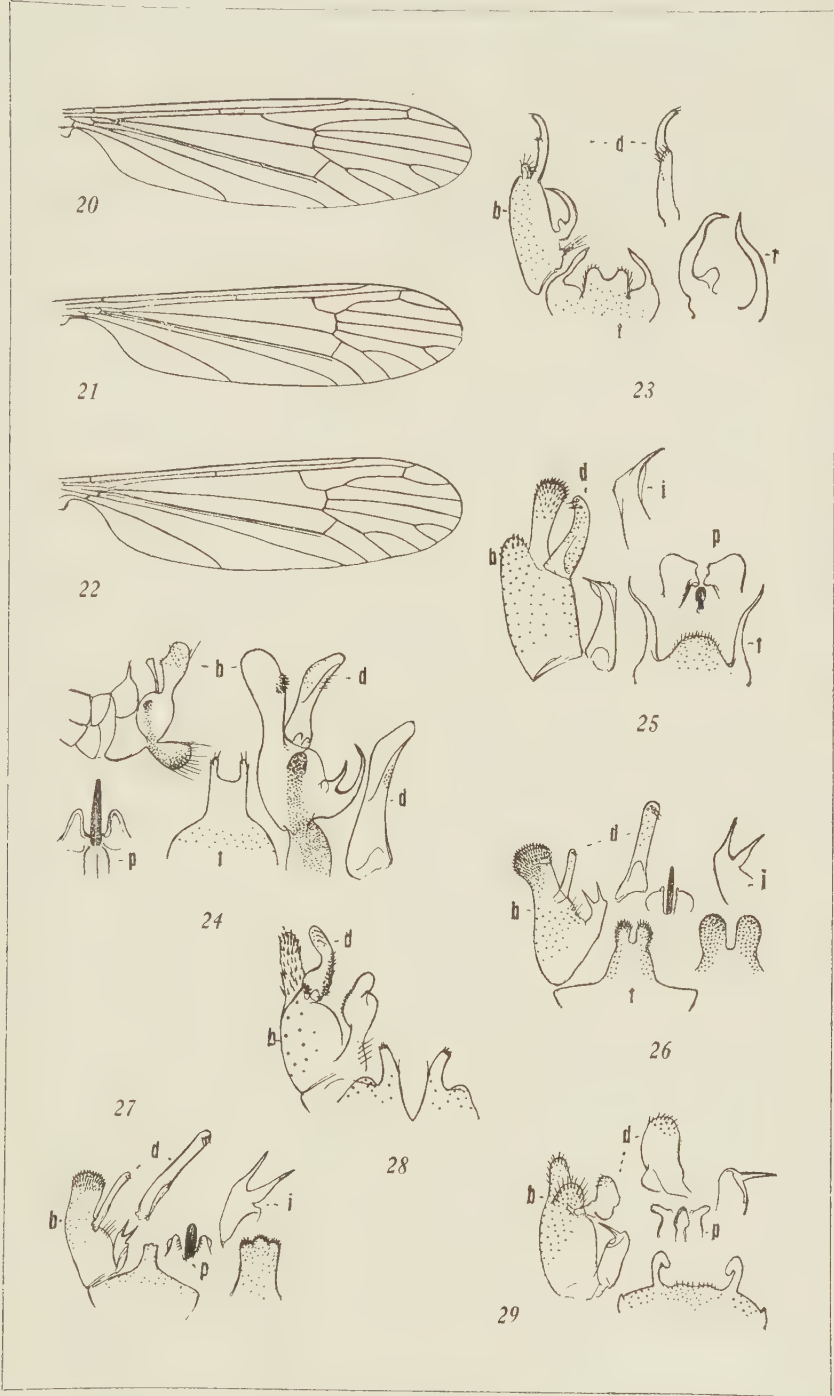
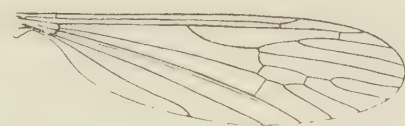


PLATE 4.



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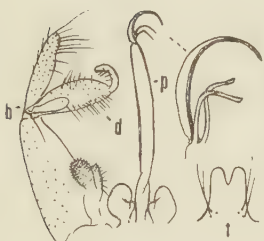
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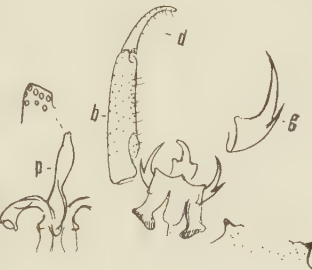
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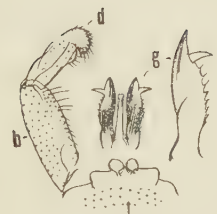
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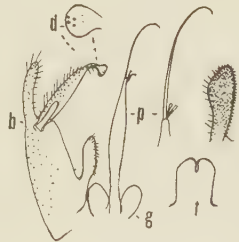
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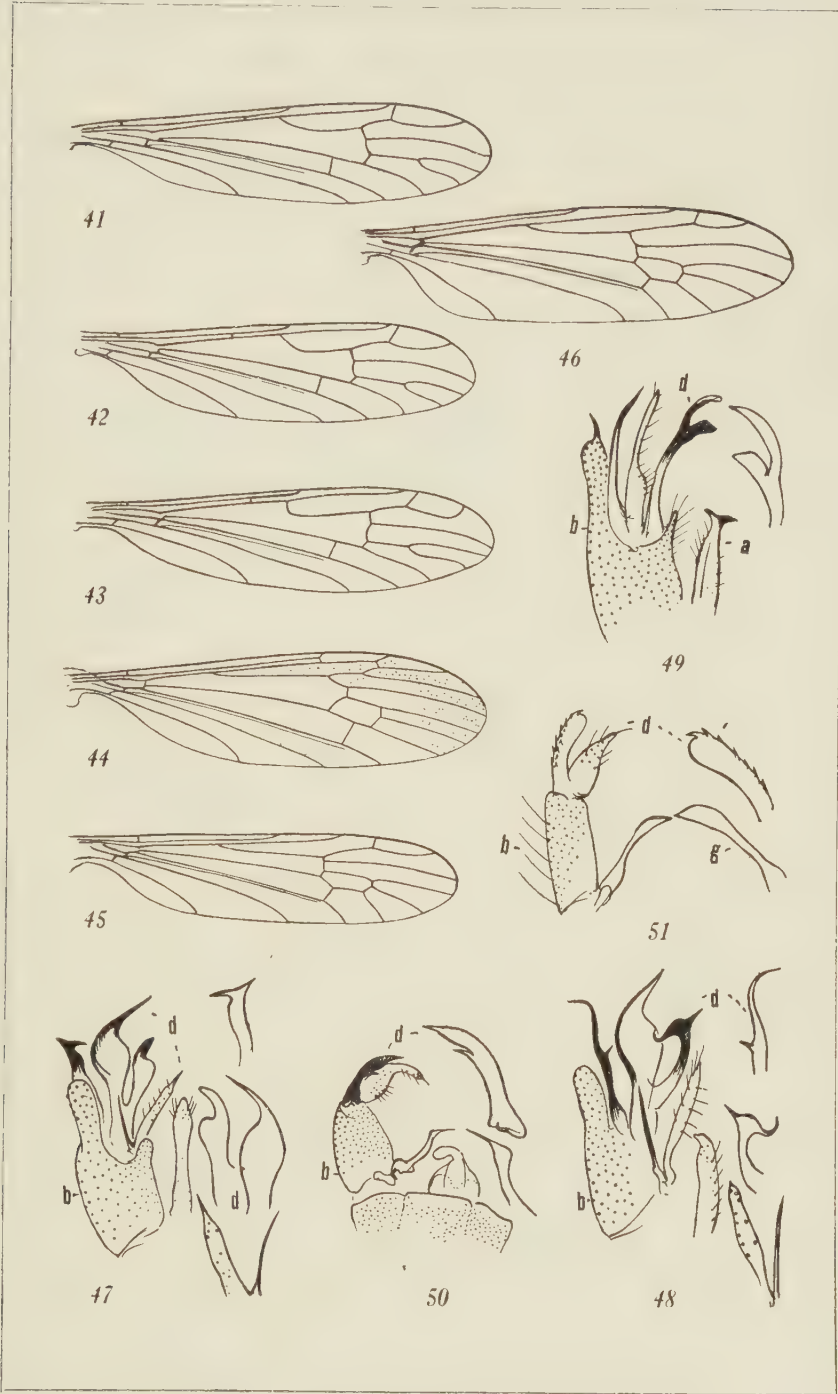


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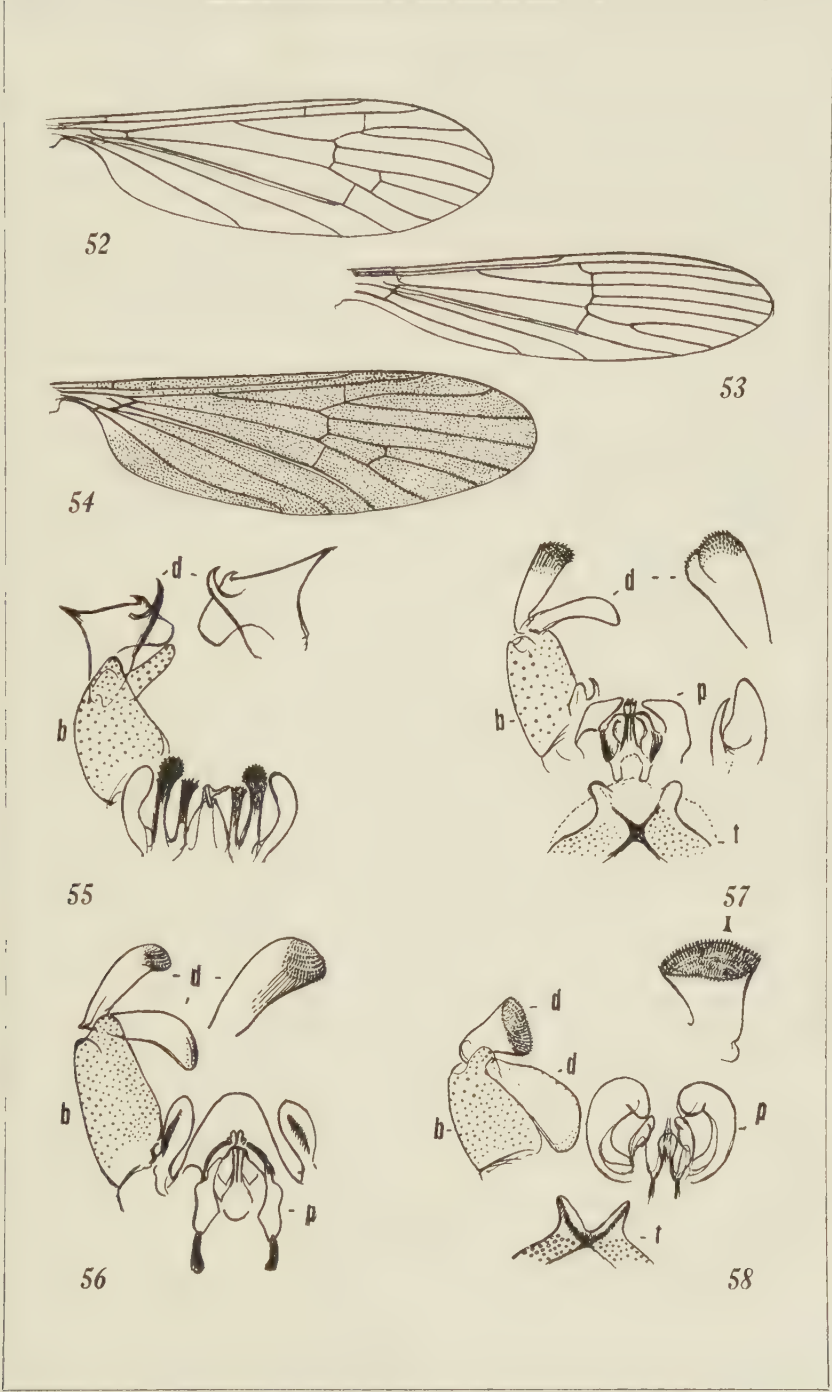
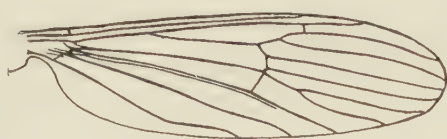
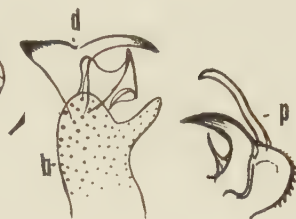


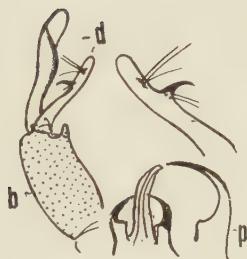
PLATE 7.



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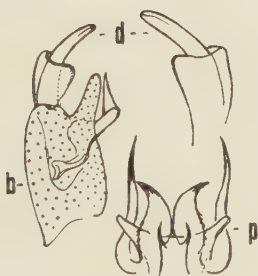
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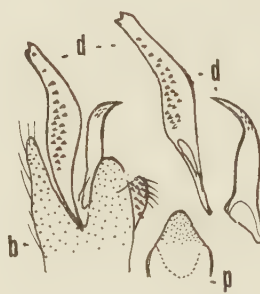
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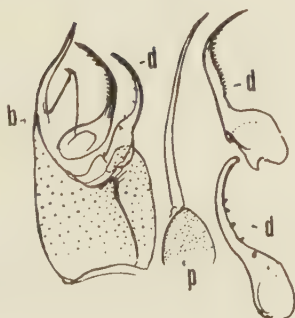
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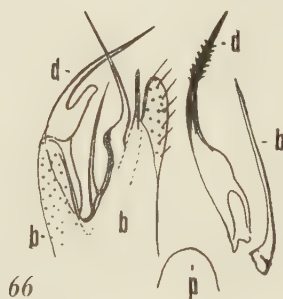
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